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# Bioenergy sensing in the brain

## The role of AMP-activated protein kinase in neuronal metabolism, development and neurological diseases

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**Key words:** AMPK, energy, neuron, brain, metabolism, glucose, neurodegenerative disease, cell death, neural development, polarization

**Abbreviations:** ACC, acetyl-CoA carboxylase; AD, alzheimer disease; AICAR, 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside; AIS, autoinhibitory sequence; AMPKK, AMPK kinase; AMPK, AMP-activated protein kinase; BRSK, brain-specific kinase; CaMKK, calcium/calmodulin-dependent kinase kinase; CBS, crystathionine β-synthase; Cdk5, cyclin-dependent kinase 5; CK1, casein kinase 1; CNS, central nervous system; GLUT, glucose transporter; GSK, glycogen synthase kinase; HD, Huntington disease; IGF1, insulin-like growth factor 1; IRS, insulin receptor substrate; LKB1, liver kinase B1; MARK, microtubule-associated protein-regulating kinase/microtubule affinity-regulating kinase; mTOR, mammalian target of rapamycin; NTD, N-terminal domain; mtTFA, mitochondrial transcription factor A; NPC, neural progenitor cell; PI3K, phosphatidylinositol-3-kinases; TAK1, TGFβ-activated kinase-1

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**Bioenergy homeostasis constitutes one of the most crucial foundations upon which other cellular and organismal processes may be executed. AMP-activated protein kinase (AMPK) has been shown to be the key player in the regulation of energy metabolism, and thus is becoming the focus of research on obesity, diabetes and other metabolic disorders. However, its role in the brain, the most energy-consuming organ in our body, has only recently been studied and appreciated. Widely expressed in the brain, AMPK activity is tightly coupled to the energy status at both neuronal and whole-body levels. Importantly, AMPK signaling is intimately implicated in multiple aspects of brain development and function including neuronal proliferation, migration, morphogenesis and synaptic communication, as well as in pathological conditions such as neuronal cell death, energy depletion and neurodegenerative disorders.**

### Introduction

The intracellular environment is rarely in a state of static equilibrium, but instead exists in a state of constant energetic flux. Since bioenergy supply constitutes the very foundation for all forms of cellular activities, it is essential for cells to be equipped with a mechanism that couples activity and energy consumption to metabolism and energy supply. To meet this demand, eukaryotic cells have evolved AMP-activated protein kinase (AMPK), a protein capable of monitoring energy expenditure and efficiently regulating energy homeostasis.

AMPK monitors cellular energy status through sensitivity to the cellular AMP:ATP ratio, becoming active in response to metabolic stresses that decrease concentrations of ATP. Once activated, AMPK utilizes its serine/threonine kinase activity to increase the rate of cellular catabolism and simultaneously inhibit anabolic processes, resulting in a net increase in ATP production. The downstream pathways regulated by AMPK present an ever growing list that includes glycolysis, fatty acid oxidation and mTOR-mediated protein synthesis.[1-3] However, AMPK's role is not limited to cell-autonomous energy regulation; many metabolic hormones, including leptin and adiponectin, function as upstream modulators of AMPK activity, allowing AMPK to participate in the maintenance of whole-body energy homeostasis. Additionally, the anti-diabetic drug metformin has also been observed to promote AMPK activity under a variety of experimental conditions.[4-7]

The importance of AMPK in general metabolic regulation has been appreciated for a long time, but its role in brain development and function has only recently been investigated. Given that the brain is the most energy-consuming organ in the body, it is logical to assume a crucial role of AMPK in neuronal energy regulation and brain activity. In this review, we will provide an overview on the general structure and regulation of AMPK before expanding on recent insights highlighting the role of the AMPK signaling cascade in

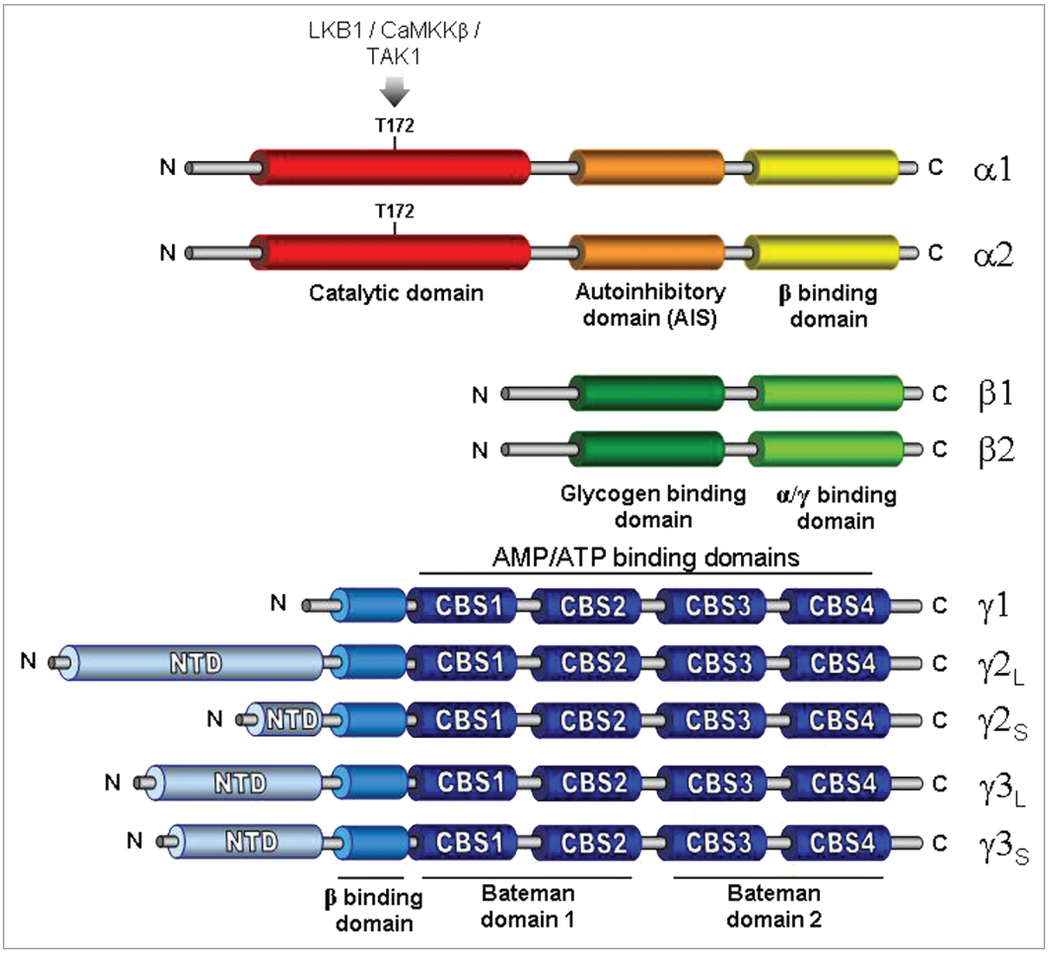


**Figure 1.** Isoforms and splice variants of mammalian AMPK. The two α subunit isoforms each contain an N-terminal catalytic domain that shares 90% homology, including a threonine residue (T172) that is phosphorylated by upstream kinases for activation. While both isoforms exist within the brain, α2 is dominantly expressed in neurons. The two β subunits contain a glycogen-binding domain that is thought to play a role in the subcellular localization of AMPK in muscle cells and a C-terminal domain required for interaction with the α and γ subunits and complex formation. Three different isoforms exist for the γ subunit (γ1, γ2 and γ3), with γ2 and γ3 each possessing two splice variants (long and short forms) that vary in the length of the N-terminal domain (NTD). γ1 is the predominant isoform within the CNS, with γ3 being restricted to skeletal muscle.

the brain, including energy metabolism, neuroprotection/toxicity and neurodevelopment as well as the involvement of AMPK activity in certain neuropathological conditions.

## AMPK Structure and Isoform Distribution

AMPK is a heterotrimeric protein complex that consists of α, β and γ subunits in equal stoichiometry. While the α subunit confers catalytic kinase activity, the β and γ subunits function in a regulatory capacity. The catalytic domain of the α subunit is located on the N-terminal region of the protein, while the C terminus is required for interaction with the β and γ subunits and complex association[8] (**Fig. 1**). Variation between mammalian α isoforms is limited to non-catalytic regions, as the N-terminal catalytic core of α1 and α2 share 90% amino acid sequence identity, whereas the remaining protein displays only 61% homology.[9] Also conserved within the α subunit is an autoinhibitory sequence (AIS) C-terminal relative to the kinase domain. The AIS sequence has been shown to repress kinase activity, as bacterially expressed constructs that contain both the kinase domain and the AIS sequence are roughly ten times less active than constructs that contain the kinase domain alone.[10,11] Conservation within the β subunit is located to a central glycogen-binding domain (GBD) and a C-terminal region that functions as a scaffolding domain, allowing its association with both α and γ subunits.[12] Mammalian γ subunits contain four tandem repeats named crystathionine β-synthase (CBS) domains, as the repeats were first identified in the enzyme crystahionine β-synthase.[13] These domains function in tandem pairs, referred to as Bateman domains, binding to one molecule of either AMP or ATP in a mutually exclusive manner.[14-17]

Multiple isoforms exist for each of the three subunits, with both the α and β subunits having two isoforms (α1, α2, β1, β2), while three known isoforms exist for the γ subunit (γ1, γ2 and γ3).[18] However, expression of γ3 is restricted to skeletal muscle.[19] A study investigating the

distribution of the various AMPK subunit isoforms in mice found that all three subunit isoforms exist in the CNS, but expression varies among different cell types. For instance, the α2 catalytic subunit is highly expressed in neurons compared with α1, which is expressed to a lesser degree in neurons. α subunits are not detected in glial cells at basal conditons, except in the activated astrocytes. The γ1 subunit is the dominant isoform expressed in neurons but is absent in astrocytes, while the levels of β1 and β2 subunit expression vary among different CNS cell types. Moreover, the expression of these isoforms within the brain is not static but, instead, displays developmental changes. It has been found that the mRNA levels for α2 and β2 subunits increase between embryonic days 10 and 14, whereas expression of α1, β1 and γ1 is consistent throughout ages E10 to P25.[20] Currently, the basis for distinct spatial and temporal AMPK subunit expression remains unknown; however, higher affinity of the β2 for glycogen, compared with that of β1, has recently been revealed, possibly explaining the predominant expression of this isoform in muscle cells.[21]

## Regulation of AMPK Activity

**Basis for molecular activation.** The ability of the Bateman domains to bind either AMP or ATP exclusively, confers the sensitivity of AMPK to the cellular AMP:ATP ratio, and ultimately allows the protein to "sense" the energy level of the cell. More specifically, the binding of AMP to the γ subunit Bateman domains promotes activation of AMPK in three distinct ways.

First, the Bateman domains bind AMP in a cooperative manner, meaning binding of AMP to the first Bateman domain enables AMP binding to the second with greater affinity. Second, AMP binding to the γ subunit produces a conformational change in the AMPK complex, exposing threonine-172 (Thr172) in an activation loop of the α subunit. Phosphorylation of Thr172 by upstream kinases, referred to as AMPK kinases (AMPKKs), cause a roughly 50–100-fold increase in AMPK activity.[22] Lastly, in addition to making AMPK a better substrate for AMPKKs, AMP binding also inhibits protein phosphatase-mediated dephosphorylation of Thr172.[23] The combination of all three activation mechanisms enables AMPK to respond to very low levels of AMP, making it highly sensitive to slight fluctuations in cellular energy levels. Important to AMPK's role as an energy sensor, Bateman domains are also capable of binding ATP in a cooperative manner, although with much lower affinity than AMP, producing an antagonistic effect on AMPK activation.[14] The inverse relationship between AMP and ATP binding in regard to AMPK activation creates a negative feedback loop, ensuring that AMPK activity decreases once cellular energy is recovered.

**Regulation of AMPK by upstream kinases.** In addition to a rise in cellular AMP:ATP ratio and subsequent AMP binding, the ability of AMPK to regulate metabolic activity relies heavily upon Thr172 phosphorylation by upstream kinases. However, the identities of the kinases responsible for AMPK α subunit phosphorylation were unknown for decades following the initial discovery of AMPK. The first clue as to the identity of potential AMPKKs came from screening studies that identified multiple upstream kinases for the yeast homolog of AMPK, Snf1.[24-26] This information was used to identify two mammalian AMPKKs: the tumor suppressor LKB1 [27,28] and the calmodulin-dependent protein kinase kinases, CaMKKα and CaMKKβ.[29-31]

LKB1 was originally acknowledged as the tumor suppressor mutated in the genetically inherited susceptibility to human cancer, coined Peutz-Jeghers Syndrome.[32] In regard to AMPK activation, studies performed in peripheral tissues demonstated that LKB1 is indeed necessary for phospho-activation of AMPK.[27,33] Despite both LKB1 and AMPK being ubiquitously expressed in mammalian cells, there is evidence to suggest that AMPK may be acted upon by different AMPKKs in a tissue-specific manner. For instance, LKB1 has been demonstrated to be the major upstream activator of AMPK in muscle[34] and liver cells;[35] however, a study utilizing LKB1-knockouts found that LKB1 deficient neurons had similar levels of phosphorylated AMPK as compared with wild-type cells under normal physiological conditions.[36] This observation suggests that another kinase may fulfill the role as the major AMPKK in the brain. To this end, Hawley et al. were able to show that in rat brain slices, intracellular increases in calcium following membrane depolarization resulted in a high degree of AMPK phosphorylation that was blocked by the CaMKK inhibitor STO-609. Because membrane depolarization did not affect the cellular AMP:ATP ratio, this study suggests that AMPK can be regulated in a $Ca^{2+}$-dependent, AMP-independent manner.[29] It will be interesting to know whether CaMKK is sufficient to phosphorylate neuronal AMPK during periods of energetic stress.

Ongoing research into identifying potential AMPKKs has revealed additional kinases in various tissues. The member of the mitogen-activated protein kinase kinase kinase family, TGFβ-activated kinase-1 (TAK1), was observed to phosphorylate AMPK in HeLa cells that lack LKB1 activity.[37] Additionally, in cardiomyocytes, dominant-negative expression and gene knockout of TAK1 caused inhibition of AMPK T172 phosphorylation. TAK1 activity was found to be required in the activation of AMPK in response to Metformin, AICAR and ischemia.[37,38] Although these studies make a strong point for TAK1 regulation of AMPK in peripheral tissues, it remains unclear whether TAK1 functions as a major regulator of AMPK in the brain.

## AMPK Regulates Glucose Transport in Neurons

The brain constitutes only 2% of the body's weight but utilizes 50% of the total glucose supply. This can be attributed to the fact that neurons are the most metabolically demanding cells. But of more relevance, unlike peripheral tissues that utilize multiple nutrient sources for energy production, neurons use only glucose as fuel. The need for steady glucose uptake is compounded by the fact that neurons do not store glycogen and, therefore, must tightly couple energy demand to glucose influx in order to ensure a stable and continuous energy supply.[39] In support of this, research has begun to emerge illustrating the relationship between AMPK activity

and various energy-consuming neuronal activities.

In the brain, glutamate is the major neurotransmitter mediating most of the synaptic transmission. Multiple molecular events occurring during synaptic activation, such as sodium pump activity, receptor trafficking, cytoskeletal rearrangements, signaling and metabolic processes, make synaptic activity an energetically costly endeavor. It is thus conceivable for a crosstalk between glutamatergic excitation and energy mobilization. In line with this point, a study by Weisova et al. shows that cerebellar granule neurons had increased levels of phosphorylated AMPK in response to glutatmate stimulation, which was accompanied by a decrease in cellular ATP concentrations,[40] explaining the basis for AMPK activation. Furthermore, glutamate excitation produced an increase in the surface expression of the glucose transporter GLUT3 that persisted for hours following the initial glutamate treatment. Importantly, increased GLUT3 surface expression was mediated by AMPK, as inhibition of AMPK, either through knockdown or pharmacological inhibition, blocked the GLUT3 response to glutamate. Interestingly, CaMKK inhibition had little effect on AMPK-mediated GLUT3 translocation, indicating AMPK activation by an alternative AMPKK during glutamate activation. Consistently, AMPK activity also translocates glucose transporters to the plasma membrane in skeletal and cardiac muscle during exercise;[41-44] these findings implicate AMPK as a crucial mediator coupling increased energy demands associated with neuronal activity and glucose uptake.

In peripheral tissues, insulin is a key signaling component in glucose uptake, while the regulation of neuronal glucose influx has traditionally been viewed as an insulin-insensitive process. However, there is evidence to suggest that both the insulin and insulin-like growth factor 1 (IGF1) signaling pathways are involved in glucose uptake in the brain.[45-47] For instance, suppressing endogenous insulin signaling in humans using somatostatin results in decreased glucose uptaking in the brain, as monitored by 18-fluorodeoxyglucose uptake and positron emission tomography (FDG-PET).[48] Likewise, insulin treatment in cultured hippocampal neurons facilitates GLUT3 membrane translocation, which requires membrane depolarization for the final fusion of GLUT3-carrying vesicles with the plasma membrane to facilitate glucose uptake.[49] Furthermore, IGF1 expression, which is closely related to insulin but shows an increased ability to cross the blood brain barrier[50] and higher mRNA expression within the brain,[51,52] was observed to parallel radiolabeled glucose uptake in mouse brains.[46] In this same study, IGF1-null mouse brains showed reduced glucose uptake, implicating a role for IGF1 signaling in the regulation of glucose uptake within the brain.

Both insulin and IGF1 bind to receptor tyrosine kinases that associate with insulin receptor substrate (IRS),[53] a known upstream activator of the PI3K/Akt signaling cascade.[54] In peripheral cells, PI3K activation plays a significant role in glucose uptake[55,56] and, therefore, may be involved in glucose influx within the brain as well. To this end, insulin increased GLUT4 translocation to the plasma membrane in human SH-SY5Y neuronal cell lines in a PI3K-dependent manner.[57] Furthermore, in mouse brains, GLUT4 was localized to neuronal processes rich in both IGF1 and phosphorylated Akt, suggesting a physical association between these two proteins. Moreover, in Igf1-null brains, GLUT4 expression as well as Akt phosphorylation were reduced, suggesting that Igf1-induced Akt phosphorylation may play a role in translocation of GLUT4 to the neuronal membrane.[46]

Given that insulin/IGF1 participate in the regulation of intracellular transport of glucose, it is reasonable to hypothesize a connection between the energy sensor AMPK and the insulin/IGF cascade. In fact, AMPK is capable of phosphorylating IRS-1, the most upstream component of the insulin/IGF1 signaling cascade, in cell-free assays and mouse myoblast C2C12 cell lines in response to AICAR.[58] In further support of this notion, our recent work showed that in both cultured rat embryonic hippocampal neurons and embryonic cortical brain slices, pharmacological activation of AMPK with the agonist AICAR resulted in a robust increase in the activity of the PI3K-Akt system.[59] Importantly, increase in pAkt was blocked by inhibition of AMPK or PI3K activity, confirming a sequential activation of PI3K and Akt downstream of AMPK. Theses findings suggest that by activation of the PI3K/Akt cascade, probably via phosphorylation of IRS-1, AMPK relays energy-lacking signals to the glucose transport system to facilitate energy production.

## AMPK Regulates Mitochondrial Function and Biogenesis

Mitochondrial function and biogenesis appear to be an important target of AMPK activation as an effort to increase cellular energy for extended periods of time. In fact, a major target of the AMPK cascade is the phosphorylation and subsequent inhibition of acetyl-CoA carboxylase (ACC),[60,61] the enzyme responsible for converting acetyl-CoA to malonyl-CoA. By reducing malonyl-CoA production, AMPK facilitates CPT1-mediated long chain fatty acyl-CoA transport into the mitochondria, where the catabolic process of fatty acid β-oxidation can then occur.[62]

Skeletal muscles, much like neurons, are subject to high metabolic demands, as they are commonly required to perform multiple contractions over short periods of time. To maintain energy stores, muscle cells upregulate mitochondrial enzymes to compensate for chronic contraction and fatigue.[63] More recently, experiments performed in skeletal muscle show that lasting contraction also results in elevated AMPK activity.[64] Furthermore, chronic subcutaneous injection of AICAR into rat quadriceps increased mitochondrial enzyme cytochrome *c*, GLUT4 expression and ATP concentration.[64] AMPK activity has also been linked to mitochondrial biogenesis, as mice expressing a dominant-negative AMPK mutant fail to increase their mitochondria mass in response to β-guanidinopropionic acid, a creatine analog that mimics endurance exercise and increases mitochondrial biogenesis in wild-type mice.[65] Mechanistically, this was found to result from the ability of AMPK to activate PGC-1α, a transcriptional co-activator that, along with the transcription factor NRF-1, upregulates nuclear genes required for mitochondrial biogenesis.[65-68]

In addition to muscle cells, recent studies show that mitochondrial biogenesis in neurons can also be regulated by AMPK. For instance, RT-PCR performed on Neuro2a cells treated with resveratrol, a polyphenol shown to activate AMPK in culture,[69] revealed increased mRNA levels for the mitochondrial protein marker mitofusin 2 and two key regulators of mitochondrial biogenesis, PGC-1α and mitochondrial transcription factor A (mtTFA).[69,70] Furthermore, the addition of the AMPK antagonist compound C, or expression of a dominant-negative AMPK mutant blocked the upregulation of these mitochondrial markers, supporting a role of AMPK in neuronal mitochondrial biogenesis. Another study showed that KCl depolarization of cultured rat visual cortical neurons activated AMPK and produced an increase in PGC-1α, NRF-1 and mtTFA levels and, furthermore, effectively increased ATP production.[71] Addition of AICAR or resveratrol to neuron cultures also resulted in increases in PGC-1α and NRF-1 mRNA levels. Importantly, these changes were abolished by suppression of AMPK activity.

## AMPK in Neurodevelopment

**Proliferation and differentiation.** Although the role of energy homeostasis in mature brain functions has been extensively investigated, energy status in early brain development remains less well-understood. The complex cellular processes occurring during this time, including neuronal differentiation, migration, neurite growth and cellular polarization, consume a vast amount of energy and demand a secured and regulated energy supply.

In this regard, it has been found that loss of AMPK activity in Drosophila results in embryonic lethality, indicating an important role for AMPK activity in neural development.[72] This study also suggests that AMPK is involved in maintaining genomic integrity during neural progenitor cell division, as a significant population of neuroblasts from AMPK-deficient larval brains displayed polyploidy.[72] Additionally, another study utilizing Drosophila as a model system illustrated that loss of AMPK activity, via β-subunit knockout also results in extensive neurodegeneration shortly after neuronal differentiation.[73]

A study examining AMPK in the mammalian brain showed that knockout of AMPK β1 (AMPK $\beta^{-/-}$) subunit in mice leads to severe loss of neurons and oligodendrocytes as well as abnormal astrocyte proliferation.[74] Loss of AMPK functionality also produced proliferative defects and increased apoptosis within neural stem cell progenitors (NPCs), resulting in a 50% smaller brain than wild-type littermates. Retinoblastoma protein (Rb), known to be required in NPCs for normal brain development,[75] was found to be directly phosphorylated by AMPK and showed hypophosphorylation in AMPK $\beta^{-/-}$ NPCs. Importantly, the misregulation of Rb was observed to prevent NPCs from exiting and reentering the cell cycle, thereby accounting for the decreased proliferation of AMPK $\beta^{-/-}$ NPCs.[74] However, a study by Zhang et al. demonstrated that AICAR treatment and glucose deprivation caused cycle arrest in $G_0$ phase and resulted in reduced proliferation of the immortalized mouse neural progenitor cell line, C17.2. This, again, was shown to result from the hypophosphorylation of Rb, an effect that was blocked by either compound C or expression of a dominant-negative AMPK.[76] The differences observed may result from variations between the in vivo and cell line models utilized by these studies, but further investigation is required to solidify the effect AMPK activity has on proliferation within the CNS.

**Polarization and migration.** Neuronal polarization constitutes the differentiation and elongation of minor neurites into multiple dendrites and a single axon. Because a neuron typically generates an axon of impressive size and complex geometry,[77] its polarization entails extensive biosynthesis of protein and lipids, active transport of building materials to the axonal growth cone and extensive cytoskeleton rearrangements.[78] These molecular processes must be sustained by a large amount of ATP consumption, suggesting potential sensitivity of neuronal polarization to cellular bioenergy status.[79]

Our recent study has demonstrated that when AMPK activity is pharmacologically enhanced to mimic energy-lacking conditions at the beginning of neuronal development, axon initiation and neuronal polarization are suppressed in both cultured embryonic hippocampal neurons and embryonic cortical brain slices, resulting in neurons that lack morphological polarization.[59] This is concluded to result from AMPK-dependent disruption of PI3K enrichment at the neurite tip, previously shown to be a critical factor in axon initiation and polarization.[80] Mechanistically, we find that direct phosphorylation of the light chain of the motor protein kinesin kif5 leads to the dissociation of the PI3K cargo from the motor complex and a failure of PI3K delivery to the neurite tip. Furthermore, we find that, although AMPK activity has a strong inhibitory effect on neuronal polarization, expression of a dominant-negative version of AMPK in both cultured neurons and brain slices has no effect on axon growth and polarization. In another study, knockout of both AMPKα1/α2 subunits in mice shows that AMPK activity is not required for cortical neurogenesis, neuronal migration or polarization under normal conditions.[81] These findings point to a negligible role for basal AMPK activity during normal polarization, implying that AMPK may work to regulate neuronal polarization under pathological energy-lacking conditions. Indeed, a brief ischemia challenge during neuronal development results in inhibition of axon initiation and polarization (Amato, 2011). Further research is needed to determine whether neurons can resume axon growth after long periods of time, although short periods are not sufficient for recovery, as neurons remain unpolarized three days after removal of AMPK activators.[59] Interestingly, LKB1, a major upstream activator of AMPK, has been shown to be required for polarization of cortical neurons in vivo.[36,82] However, LKB1-deficient cortical neurons showed no change in phosphorylated or total AMPK levels, indicting that the effect of LKB1 in neuronal polarization is independent of AMPK activity.[29]

## AMPK in Neuronal Viability

Many of the cellular insults that decrease cellular energy, such as ischemia and

hypoxia, are known to induce apoptosis.[83,84] It is thus not surprising to discover the involvement of AMPK activity in cell survival in both peripheral cells and the CNS. In *C. elegans,* activity of the LKB1/AMPK pathway is required for conservation of lipid reserves and thereby ensures survival of the larvae through maturation.[85] Also, AMPK activity was observed to block TNFα-induced cardiomyocyte apoptosis that normally results from insults such as myocardial infarction and ischemia reperfusion,[86] suggesting a neuroprotective role of the kinase.

In neurons, conflicting findings have been reported regarding the role of AMPK in cell death. AMPK activity appears to be both neuroprotective and pro-apoptotic, probably due to differences in nerve cell types, the nature of neural insults and the intensity and length of AMPK activation. For instance, pharmacological activation of AMPK via AICAR treatment effectively enhanced hippocampal neuron survival in response to glucose deprivation, chemical ischemia and glutamate exposure. Importantly, knockdown of the α1 and α2 subunits abolished the beneficial effects of AICAR, indicating direct involvement of AMPK activity.[87] The neuroprotective effect may be mediated by neuronal inhibition. In response to ischemic insult, AMPK was observed to phosphorylate the R2 subunit of the $GABA_B$ receptor, a G protein-coupled receptor involved in post-synaptic hyperpolarization, resulting in enhanced receptor activity. AMPK-mediated upregulation in $GABA_B$ receptor activity has been shown to lead to post-synaptic hyperpolarization, reduced excitotoxicity and improved neuronal survival after chemical anoxia.[88]

Conversely, a study revealed that while AMPK activity significantly increased in response to ischemia, pharmacological inhibition of the kinase reduced stroke damage. Consistently, activation of AMPK through AICAR treatment intensified stroke damage in mice.[89] Furthermore, the same study showed that knockout of AMPK α2 but not α1 subunit significantly reduced stroke-caused cell death, consistent with the results from AMPK inhibition.[90]

While knockout of AMPK activity in these experiments implicate direct involvement of AMPK, the wide-ranging effects AMPK imposes on cell survival begs further experimentation. It has been hypothesized that perhaps the difference between in vitro and in vivo findings stems from discrepancies in glucose supply, with glucose concentration in neuronal culture medium roughly ten times of that found at physiological conditions.[91] Additionally, studies utilizing AICAR treatment showed that incubation time was also a factor in AMPK's effect on cell survival.[92] Obviously, it is important to understand the molecular details underlying the different or opposite effects of AMPK on neuronal survival, so that a precise manipulation on AMPK signaling cascade can be designed for tentative clinical treatment.

## AMPK in Neurodegenerative Diseases

AMPK is implicated in many metabolic diseases, including diabetes, obesity and cardiovascular disease. Recent studies have also revealed an important role of AMPK in neurodegenerative diseases, particularly Alzheimer disease (AD).[93] AD is known to be associated with abnormalities in energy metabolism, including decreased glucose uptake and insulin sensitivity, compromised mitochondrial activities and impaired lipid metabolism.[94,95] Hypometabolism and ATP depletion can cause partial membrane depolarization due to reduced sodium pump efficiency, which will potentiate NMDA receptor activity and eventually lead to neurotoxicity and cell death.[96] Amyloid β (Aβ), the key molecule in AD pathogenesis, is a product of cleavage of its precursor protein APP by β-secretase (BACE1) and γ-secretase. It has been shown that in primary cortical neurons and N2a neuroblastoma cells, incubation with the AMPK activator metformin leads to an increase in both intracellular and extracellular Aβ generation. This effect is mediated by an elevated expression of BACE1 and abolished by AMPK inhibitor compound C, strongly indicating the direct role of AMPK in Aβ production.[97] In contrast, a recent study showed that in cultured rat cortical neurons, activation of AMPK by AICAR treatment caused a significant reduction in Aβ. Consistently, AMPK α2 knockout led to increased Aβ production.[98] Although possibly due to the use of a different AMPK activator or the intensity of AMPK activation, the exact reasons for the conflicting observations are not clear. The inhibitory effect of AMPK on amyloid production is supported by another study showing that activation of AMPK, either directly by AICAR treatment or indirectly via CaMKKβ following resveratrol incubation, lead to a reduction in Aβ levels.[99] This finding illustrates AMPK signaling as the downstream event in resveratrol-induced Aβ down-regulation.[100] In addition, AMPK cascade also regulates Aβ degradation and removal from neurons.[101]

Hyperphosphorylaiton of microtubule-associated protein tau is another hallmark of AD. To date, more than 30 protein kinases have been implicated in tau phosphorylation, in which GSK3β (glycogen synthase kinase 3), Cdk5 (cyclin-dependent kinase 5), CK1 (casein kinase 1) and PKA play more important roles.[102,103] In line with metabolic disregulation in AD, AMPK has recently been identified as a novel kinase targeting tau for protein phosphorylation. AMPK can phosphorylate tau at multiple sites, including $Ser^{262}$, $Ser^{356}$ and $Ser^{396}$, reducing the interaction of tau with microtubules.[104] Interestingly, this work indicates that Aβ itself can cause AMPK activation via regulation of NMDA receptor-dependent intracellular calcium homeostasis and CaMKKβ activity, indicating the existence of a complex, intertwined regulatory network in the development of AD.[104] Furthermore, other AMPK family members, including MARK (microtubule-associated protein-regulating kinase/microtubule affinity-regulating kinase) and BRSK (brain-specific kinase) have also been shown to be involved in tau phosphorylation.[104-106]

Although studies on the involvement of AMPK cascade in other neurodegenerative disorders are limited, its role does not seem limited to AD. For instance, in humans and mice with Huntington disease (HD), AMPK α1 subunit was selectively activated and enriched in the nucleus of striatal neurons. Overactivation of AMPK enhanced

neuronal death and facilitated formation of Huntington aggregates. Importantly, suppression of AMPK activity or blocking its nuclear localization showed neuroprotective effects.[107] AMPK activation is also observed in a paradigm of Parkinson disease (PD) induced by application of MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine).[108] Furthermore, AMPK may be implicated in neurodegenerative disorders through general cellular mechanisms. For example, dysfunction in protein degradation is a common feature shared by most neurodegenerative diseases, which leads to the formation of intracellular aggregates and neuronal toxicity. In supporting this notion, AMPK is a major player in the regulation of autophagic process in neurons via inhibition of mTOR pathway or activation of ATG1 enzyme.[109,110]

## Concluding Remarks

As a master regulator of bioenergy status, AMPK has gained a growing amount of attention in its roles in numerous physiological and pathological systems. Its importance in neural tissue is especially exceptional, given that the brain is the most energy-demanding organ in the body and is therefore the most vulnerable to energy disregulation and depletion.[111] AMPK is critically involved in multiple developmental stages and functionality of neurons, including migration, maturation, glucose uptake and metabolism and neuronal communication, including synaptic transmission[112] and plasticity.[113] At a system level, AMPK localized in energy-sensing neurons and circuits in the hypothalamus is responsible for the control of feeding behavior by incorporating peripheral energy-related signals.[114-117] It is worthy to note that at least 12 AMPK-related enzymes exist that show homology in the catalytic domain, constituting a AMPK-related kinase family that includes BRSK 1–2, NUAK 1–2, SIK 1–3, MARK 1–4 and MELK,[118] but their functions remain largely unknown. We predict a rapid expansion in the investigation of AMPK and its related kinases, especially on their crucial roles in the formation and function of the nervous system


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