Review

The role of neuronal AMPK as a mediator of nutritional regulation of food intake and energy homeostasis

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ABSTRACT

Hypothalamic 5′-adenosine monophosphate-activated protein kinase (AMPK) senses intracellular metabolic stress, i.e., an increase in the cellular AMP:ATP ratio, and integrates diverse hormonal and nutritional signals to restore energy balance. Recent evidence suggests that different nutrients can modulate AMPK activity in the hypothalamus, thereby controlling weight gain through a leptin-independent mechanism. Understanding the mechanisms by which nutrients control hypothalamic AMPK activity is crucial to the development of effective nutritional interventions for the treatment of food intake-related disorders, such as anorexia and obesity. This article highlights the current evidence for the intricate relationship between nutrients and hypothalamic AMPK activity.

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1. Introduction

The increasing prevalence of food intake disorders is widely regarded as a major public health issue [1,2]. Different diets are currently used as an essential part of the treatment of food intake-related disorders, such as anorexia and obesity. Linus Pauling in the mid 1970s demonstrated the beneficial role of certain nutrients in cancer patients [3–5]. In the same decade, the Atkins diet books were sold, making Atkins food products popular. Over the years, researchers have shown that an Atkins-like diet could be beneficial for the treatment of obesity and anorexia–cachexia syndrome [6,7]. Although accumulating evidence indicates that nutrients and diets are crucial to the treatment of anorexia–cachexia syndrome and obesity, physicians, nutritionists, and physiologists are still attempting to develop new diets based on a better understanding of how nutrients modulate the physiological circuits responsible for the control of food intake and energy homeostasis.

In the central nervous system (CNS), the hypothalamus is the region of the brain that regulates food intake and energy...
homeostasis. The hypothalamus contains several nuclei, including the arcuate nucleus (ARC), the paraventricular nucleus, the ventromedial nucleus, the dorsomedial nucleus, and the lateral hypothalamic area (LHA). Several of these areas are responsible for the production of orexigenic and anorectic neuropeptides that regulate food intake and energy expenditure. The ARC produces the orexigenic neuropeptides known as agouti-related protein (AgRP) and neuropeptide Y (NPY), and the anorectic neuropeptides known as α-melanocyte-stimulating hormone and cocaine- and amphetamine-related transcript (CART). The LHA produces the orexigenic neuropeptides known as melanin-concentrating hormone and orexins, as well as the anorexigenic neuropeptide CART [8–11]. Recently, a number of neuronal signaling pathways in the hypothalamus have emerged as potential modulators of neuropeptide expression and energy balance [12–18].

One of the main intracellular proteins that integrate nutritional and hormonal signals in the CNS is 5′-AMP-activated protein kinase (AMPK) [15,19–23]. Modulation of hypothalamic AMPK can result in the altered expression of orexigenic neuropeptides (NPY and AgRP) and anorexigenic neuropeptides (POMC and CART) in the ARC nucleus. Since AMPK has been shown to play a key role in the control of food intake and body weight regulation, it is an attractive therapeutic target for the treatment of energy homeostasis disorders and other related diseases, such as metabolic syndrome and cancer-related anorexia [12,15,20,22,24–28]. This review focuses on the role of hypothalamic AMPK in energy balance, with a particular emphasis on nutrients and other bioactive food constituents that have been shown to modulate AMPK activity.

1.1. AMPK in the control of food intake

AMPK was first described in the mid to late 1980s [29]; however, several groups of researchers had discovered it more than a decade earlier [30,31]. AMPK is a heterotrimeric enzyme complex with one catalytic (α1 or α2) and two regulatory (β1 or β2 and γ1 or γ2 or γ3) subunits [10]. The conventional serine/threonine kinase activity of AMPK is mediated by the α subunit, which is activated by the phosphorylation of a threonine residue (Thr172) [32] (Fig. 1).

Hormones classically related to the control of food intake and energy balance, such as leptin, adiponectin, ghrelin, and tetraiodothyronine (T4)-thyroid, modulate hypothalamic AMPK to produce their effects [20,24,26,33,34]. Furthermore, fasting has been shown to increase hypothalamic AMPK activity, while feeding inhibits it activity [20,35]. In contrast to its effects in peripheral tissues, leptin suppresses AMPK-α2 activity in the hypothalamus, resulting in hypophagia [20,36]. In the hypothalamus, the anorexigenic effects of the inhibition of AMPK activity are mediated by at least two mechanisms: (i) the activation of acetyl-CoA carboxylase (ACC), which converts acetyl-CoA into malonyl-CoA (high malonyl-CoA levels inhibit carnitine palmitoyltransferase-1 (CPT1), which catalyses β-oxidation, resulting in the translocation of fatty acids across the mitochondrial membrane [37,38]); and (ii) the activation of the mammalian target of rapamycin (mTOR) and phosphorylation of p70S6 kinase (p70S6K) and 4E-binding protein 1 (4EBP1) [14,15,22,39]. The activation of these pathways culminates in the inhibition of orexigenic neurons (AgRP/NPY) and the activation of anorexigenic neurons (POMC/CART) [20] (Fig. 2).

Pharmacological activators of AMPK, such as AICAR, metformin and 2-deoxy-d-glucose reverse tumor-induced anorexia by increasing AMPK activation and reducing the POMC mRNA levels [12]. However, in an opposite fashion, metformin and AICAR inhibit low glucose-induced AMPK phosphorylation in primary cultures of rat hypothalamic neurons [40] and metformin sensitizes the hypothalamus to leptin to induce a phosphorylation decrease of AMPK [41]. These contradictory results may be related to the dramatic different metabolic conditions that the neurons are exposed to.

1.2. Nutrients that modulate hypothalamic AMPK

Beyond AMPK modulation by hormones, this protein is key to the nutrients’ control of energy homeostasis. In recent decades, several research groups have identified how high-fat diets, nutrients, and bioactive food constituents may modulate the regulation of food intake in the CNS, in particular in the hypothalamus (Fig. 2 and Table 1).

1.3. Macronutrients

1.3.1. Leucine and high-protein diet

Recently, convincing evidence has emerged that high-protein diets increase thermogenesis and satiety compared to lower protein diets [42]. The amount of available evidence also suggests that high-protein meals or those rich in branched-chain amino acids lead to reduced energy intake by acting on the CNS in a specific amino acid-dependent manner [15,43–45].

The mechanism by which these amino acids regulate the CNS circuits has been recently unraveled. In the CNS, the mTOR complex 1 (mTORC1) acts as an essential intracellular target of nutrients to modulate food intake and energy expenditure [46]; mTORC1 may also impair leptin signaling, increasing food intake and body weight [47]. Conversely, central administration of leucine increases hypothalamic mTOR signaling in the ARC, reduces NPY expression, and decreases food intake and body weight [15,22]. Xu et al. [48] recently proposed that leucine stimulates the mTOR pathway, in part by serving both as a mitochondrial fuel through oxidative carboxylation and as an allosteric activator of glutamate dehydrogenase. This hypothesis supports the idea that leucine modulates mTOR function, in part by regulating mitochondrial function and AMPK activity. In fact, AMPK activation inhibits the mTOR kinase signaling pathway. mTOR

Fig. 1 – Schematic representation of AMP-activated protein kinase (AMPK). AMPK is a heterotrimeric molecule that is composed of one catalytic (α) and two regulatory subunits (β and γ). The phosphorylation of the Thr172 within the α catalytic subunit activates AMPK kinase activity.
activity is inhibited by AMPK activation of the TSC1–TSC2 complex, which inactivates Rheb GTPase [14,15].

Interestingly, ATP levels are increased and the AMP:ATP ratio is decreased in the hypothalamic tissue of rats after an ICV infusion of leucine, which is associated with a reduction in hypothalamic AMPK activity [15]. The modulation of AMPK activity and the mechanisms whereby leucine decreases AMPK phosphorylation in hypothalamus are similar to those observed in C2C12 cells and muscles that also showed a decrease in the AMP/ATP ratio in parallel with a reduction in AMPK activity [49–51]. The increase in ATP levels induced by leucine is probably mediated by both its conversion to ketoisocaproate and subsequent oxidation as well as by inducing allosteric activation of glutamate dehydrogenase [48,52]. A high-protein diet exerts the same effect as leucine, decreasing AMPK and increasing mTOR activity in the hypothalamus, resulting in a reduction of food intake and weight loss [15]. Leucine seems to be the main modulator of the AMPK and mTOR pathway in high-protein diets. Thus, AMPK and mTOR interact in the hypothalamus to regulate feeding in a leucine-dependent manner during administration of a high-protein diet [15].

1.4. Glucose

In the mid 1950s, the glucostatic theory proposed reports that glucose receptors are present in the hypothalamus. In summary, this theory stated that blood glucose levels are recognized by glucose sensors in the brain and that glucose utilization modulates meal initiation [53].

Glucose sensors are located in the hypothalamus (VMH and LHA) [54,55], the nucleus of the solitary tract, and the amygdala [56,57], which are areas known to control energy homeostasis [17]. ICV administration of glucose has been shown to decrease the AMP:ATP ratio [58] and decrease hypothalamic AMPK phosphorylation and activity in fasted rats [59]. Wolfgang et al. [60] showed that hypothalamic malonyl-CoA levels depend on the carbohydrate content of the diet consumed after food deprivation. Moreover, increased glucose flux through the CNS/hypothalamus causes decreased AMPK phosphorylation and thereby increased ACC activity, malonyl-CoA, and fatty acid synthesis.

In ex vivo hypothalamic cultures, high glucose concentrations decrease the levels of both AgRP and NPY total mRNA [61]. Moreover, when hypothalamic cells expressing POMC (N-43/S) were exposed to an increased concentration of glucose, [62] the neuron demonstrated low AMPK activity. Claret et al. [25] showed that the lack of AMPKα2 in AgRP neurons in the ARC nucleus of mice leads to diminished body weight whereas lack of AMPKα2 in POMC neurons leads to increased body weight. Additionally, mice deficient in the AMPKα2 in POMC or AgRP neurons in the ARC nucleus are insensitive to anorexigenic glucose effects. Therefore, glucose-
induced decreases in hypothalamic AMPK activation have been shown to undergo neuron-specific regulation. Recently, it was also demonstrated that AMPK suppression by glucose is mechanistically involved in glucose sensing of the hypothalamic hypoxia-inducible factor pathway in POMC neurons [63].

1.5. Saturated and polyunsaturated fatty acids

Obesity is a metabolic disturbance linked with increased consumption of saturated fatty acids, which is among the most important environmental factors predisposing people to obesity in modern societies. Recent studies have shown that these fatty acids lead to hypothalamic inflammation and dysfunction in two main anorexigenic hormones, insulin and leptin, leading to impaired hypothalamic neuropeptide modulation and consequently increasing the food intake [17,64–67]. The mechanisms involved in fatty acid-induced inflammation are emerging and comprise the fatty acid induction of pro-inflammatory cytokines through activation of membrane receptors, such as toll-like receptors (TLR4 and TLR2), IL-1R and TNFR [18,68–71]. It is interesting to note that high-fat diet also leads to impaired leptin-induced hypothalamic AMPK inhibition contributing to the leptin resistance observed in obesity [72].

On the other hand, it was demonstrated that fatty acids administered via ICV could directly regulate food intake. ICV infusion of oleic acid (a monounsaturated fatty acid; n-9) diminished food intake, via a reduction in NPY mRNA and an increase POMC neuron excitability, resulting in decreased body weight [73–75]. The hypothesis that polyunsaturated (PUFA) may also modulate hypothalamic AMPK levels is attractive and deserves further investigation. Consistent with this hypothesis a Chinese study [76], showed that animals fed with high-fat diet rich in n-3 and n-6, exhibited decreased weight gain and hypothalamic NPY and AMPK-α2 mRNA levels. Moreover, a recent study also observed a reduction of hypothalamic AMPK phosphorylation levels in docosahexaenoic acid-enriched diet fed rats when compared to chow-diet [77]. Therefore, these results suggest a promising role of fatty acids in the modulation of food intake-related disorders.

1.6. Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a fatty acid with two conjugated double bonds and an 18-carbon PUFAs, and it is naturally found in meat and dairy products [78,79]. CLA presents 28 isomers; however, the major CLA isomers found in supplements are the cis-9, trans-11 and the trans-10, cis-12 isomers. The role of CLA in the regulation of energy homeostasis has been studied [80], and a significant decrease in caloric intake in mice [82] and a reduction in white adipose tissue weight in both humans and mice were observed [83]. In addition, CLA ICV administration is associated with decreased levels of both AMPK and orexigenic neuropeptides, such as NPY and AgRP in the hypothalamus [84,85].

1.7. Natural compounds

1.7.1. α-Lipoic acid

α-Lipoic acid, also known as 1,2-dithiolane-3-pentanoic acid, is a naturally occurring compound that is synthesized in small amounts by plants and animals, including humans [86,87]. The main α-lipoic acid food sources are kidney, heart, liver, spinach, and broccoli [88]. Several beneficial properties of α-lipoic acid have been reported [89,90], including its ability to act as an antioxidant and a stimulator of glucose uptake via AMPK and glucose transporter 4 (GLUT4) in muscle. It has

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Table 1 – Role of both nutrients and foods that inhibit hypothalamic AMPK activity.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal models</th>
<th>Nutrient/diet</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cota et al. (2006)</td>
<td>Rats</td>
<td>L-leucine ICV (0.2 μg of in 2 μL)</td>
<td>↑ mTOR signaling and reduces food intake via inhibition of hypothalamic AMPK</td>
</tr>
<tr>
<td>Ropelle et al. (2008)</td>
<td>Rats</td>
<td>Leucine (50 g/kg) supplemented diet and high-protein (50% protein-enriched) diet (HPD)</td>
<td>Leucine and HPD ↓ AMPK and ↓ mTOR activity in the hypothalamus, leading to ↓ NPY and ↑ POMC mRNA levels</td>
</tr>
<tr>
<td>Minokoshi e al. (2004)</td>
<td>Mice</td>
<td>Administration of IP or ICV glucose</td>
<td>↓ AMPKα-2 activity in all hypothalamic regions</td>
</tr>
<tr>
<td>Cha et al. (2008)</td>
<td>Mice</td>
<td>ICV injections (400 μg) of glucose</td>
<td>↓ AMPK protein ratio and ↓ PAMK. Moreover, ↓ POMC and ↓ CART mRNAs and ↓ NPY and AgRP expression compared with control or fructose-treated animals</td>
</tr>
<tr>
<td>So et al. (2009)</td>
<td>Mice</td>
<td>trans10 and cis12 CLA for 27 days</td>
<td>↓ weight gain and hypothalamic NPY and AMPK-α2 mRNA</td>
</tr>
<tr>
<td>Jia et al. (2009)</td>
<td>Rats</td>
<td>High-fat diet rich in n-3 and n-6</td>
<td>↓ hypothalamic AMPK activity and causes significant ↓ weight by ↓ food intake and ↓ energy expenditure</td>
</tr>
<tr>
<td>Gomez-Pinilla &amp; Ying (2004)</td>
<td>Rats</td>
<td>Docosahexaenoic acid-enriched diet</td>
<td>↓ hypothalamic AMPK activity and ↓ food intake, while ↓ AMPK and ↓ ACC and ↓ p70S6K and 4EBP1 phosphorylation</td>
</tr>
<tr>
<td>Kim et al. (2004)</td>
<td>Rats</td>
<td>α-lipoic acid (3 μg) ICV (3rd ventricule)</td>
<td>↓ food intake, body mass, fat mass and plasma insulin and leptin levels</td>
</tr>
<tr>
<td>Ropelle et al. (2008)</td>
<td>Rats</td>
<td>ICV administration of α-lipoic acid (3 μg)</td>
<td>↓ hypothalamic AMPK phosphorylation and ↓ ACC levels, leading to ↓ malonyl-CoA and ↓ food intake and body weight</td>
</tr>
<tr>
<td>Cheng PY et al. (2011)</td>
<td>Rats</td>
<td>α-lipoic acid (200 mg/kg once daily by gavage for seven weeks)</td>
<td>↓ hypothalamic AMPK phosphorylation and ↓ ACC levels, leading to ↓ malonyl-CoA and ↓ food intake and body weight</td>
</tr>
<tr>
<td>Kim et al. (2009)</td>
<td>Mice</td>
<td>Berberine ICV (5 μg)</td>
<td>↓ hypothalamic AMPK phosphorylation and ↓ ACC levels, leading to ↓ malonyl-CoA and ↓ food intake and body weight</td>
</tr>
</tbody>
</table>

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recently been shown that administration of α-lipoic acid improves the short-term insulin sensitivity and plasma lipid profile of obese patients with impaired glucose tolerance [91]. In addition, α-lipoic acid administration suppresses food intake in genetically obese Otsuka Long-Evans Tokushima Fatty rats by inhibiting hypothalamic AMPK phosphorylation after 1 h ICV administration and 30–60 min after intraperitoneal (IP) administration. Although both α1 and α2 isoforms of the AMPK-α subunit have been shown to exist in the CNS [7,59,92], only AMPK-α2 activity was reduced after α-lipoic acid administration. Additionally, the effects of α-lipoic acid on the control of food intake and obesity were accompanied by a reduction in plasma glucose, insulin, free fatty acid, and leptin levels, as well as an increase in energy expenditure and UCP-1 expression in brown adipose tissue [59].

The anti-obesity effects of α-lipoic acid are also observed in different animal models. In exercised rats, the ICV injection of α-lipoic acid decreased food intake [14]. Comparison of the α-lipoic acid-treated groups (control vs. exercise) revealed that, in exercised animals, α-lipoic acid decreased phosphorylation of both AMPK and ACC. Moreover, α-lipoic acid increased the mTOR signaling pathway, a phenomenon that was more evident in exercised animals. Treatment with α-lipoic acid in ovariectomized rats reduced hypothalamic AMPK and ACC phosphorylation, as well as food intake, body mass, fat mass, and plasma insulin and leptin levels [93]. Thus, several studies have shown that α-lipoic acid exerts potent anti-metabolic syndrome effects and decreases food intake by suppressing hypothalamic AMPK activity and increasing p70S6K/4EBP1 phosphorylation [7,14,59,90].

1.7.2. Berberine

Berberine is a naturally occurring compound present in many herbs and is one of the main alkaloids present in Rhizoma coptidis, a plant that has been used for the treatment of diabetes for more than 1400 years in China [94]. Berberine has been shown to have a variety of effects, including anti-cancer properties in rats [95]; it also improves endothelial function in humans [96] and prevents obesity, dyslipidemia, and metabolic syndrome via alterations in AMPK activity [61,97–99].

Berberine has been reported to pass the blood–brain barrier and reach the brain [97]. Berberine ICV administration has been shown to decrease hypothalamic AMPK and increase ACC activity, provoking an increase in hypothalamic malonyl-CoA levels and energy expenditure as well as a decrease in food intake and body weight in both lean and obese mice models [100]. Interestingly, Kim et al. [100] showed that ICV berberine affects several mediators of fatty acid oxidation in the muscle tissue of ob/ob mice, such as CPT-1, PPARα, FGC-1α, mCAD, and UCP3; according to the authors, these results are dependent on the reduction of both AMPK and ACC phosphorylation in the muscle. Moreover, the anorexigenic effect of berberine is associated with the reduction of AgRP mRNA and an increase in POMC mRNA levels in neurons [100].

2. Conclusion

PUFA, CLA, α-lipoic acid and glucose reduce food intake via inhibition of neuronal AMPK, whereas leucine or high-protein diet reduces food intake by inhibiting AMPK and activating mTOR signaling. Therefore, this review summarizes the emergence of hypothalamic AMPK as one of the key signal regulators for nutrient modulated energy homeostasis.

Authors contributions

GDP and JBCC performed the design of review, researched and discussed the articles and written the paper. ERR also researched the papers included in review and wrote the paper and GZR researched and discussed the papers mentioned. All authors read and approved the final version of the manuscript.

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Conflicts of interests

The authors declare that they have no competing interests.

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