REVIEW

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Hypothalamic AMPK and energy balance

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Abstract

AMP-activated protein kinase (AMPK) is the main cellular energy sensor. Activated following a depletion of cellular energy stores, AMPK will restore the energy homoeostasis by increasing energy production and limiting energy waste. At a central level, the AMPK pathway will integrate peripheral signals (mostly hormones and metabolites) through neuronal networks. Hypothalamic AMPK is directly implicated in feeding behaviour, brown adipose tissue (BAT) thermogenesis and browning of white adipose tissue (WAT). It also participates in other metabolic functions: glucose and muscle metabolisms, as well as hepatic function. Numerous anti-obesity and/or antidiabetic agents, such as nicotine, metformin and liraglutide, are known to induce their effects through a modulation of AMPK pathway, either at central or at peripheral levels. Moreover, the weight-gaining side effects of antipsychotic drugs, such as olanzapine, are also mediated by hypothalamic AMPK. Therefore, considering hypothalamic AMPK as a therapeutic target in metabolic diseases appears as an interesting strategy due to its implication in feeding and energy expenditure, the two sides of the energy balance equation.

KEYWORDS
AMP-activated protein kinase, brown adipose tissue, food intake, hypothalamus, obesity

1 | ENERGY SENSING IS NEEDED BY LIVING ORGANISMS

Through their ability to exchange energy with their environment, living organisms can survive under different conditions. Within cells, numerous metabolic pathways are implicated in the production and utilization of energy. Heterotroph species, including mammals, collect energy through the oxidation of organic compounds—essentially carbohydrates, lipids and proteins—releasing energy that will be stored as ATP. Each cell of a living organism can be considered to contain an energy-storing *"battery"* mainly composed of ATP and ADP (ATP $\leftrightarrow$ ADP + P). Draining this energy battery leads to an increase in intracellular ADP levels. Due to the reversibility of the reaction (2ADP $\leftrightarrow$ ATP + AMP), an increase in ADP:ATP ratio during energy consumption process induces a rise in AMP levels. Therefore, low intracellular energetic levels are generally coupled to high AMP concentrations. In this sense, a functional and effective intracellular energy gauge would be driven by the evolutionary conserved principle of ADP:ATP and AMP:ATP ratio sensing.[1-4]

In 1987, *David Carling and Grahame Hardie* established for the first time that the two protein kinases, implicated in the inhibition of enzymes responsible of *de novo* fatty acid and cholesterol synthesis (acetyl-CoA carboxylase, ACC and hydroxymethylglutaryl-CoA reductase, HMGCR, respectively), were actually the same protein.[5] As each

enzyme had been previously identified to be activated by AMP,[6,7] the term of *AMP-activated protein kinase (AMPK)* was suggested to identify both of them.[8] Ten years later, the same authors first proposed the role of AMPK as a master cellular energy gauge.[9] AMP-activated protein kinase is now recognized as being the main energy indicator in eukaryotic cells, which is in my view one of the most significant discoveries in biomedical sciences in the last decades. This theory was later expanded to a more global approach in which AMPK could be implicated in the regulation of numerous processes at cellular and whole-body levels, such as cell growth, apoptosis, mitosis, autophagy, cell polarity, immune function, inflammation and cancer.[4,10-12]

2 | AMPK IS THE UNIQUE AND REAL ENERGY SENSOR

AMP-activated protein kinase (AMPK) is a highly evolutionary conserved serine/threonine kinase. Numerous orthologues of the AMPK subunits have been described in the different eukaryotic species, including protists, plants, fungi and animals.[1,10] AMPK is a heterotrimer complex composed of (a) a catalytic α subunit (of which it exists two variants, α1, α2), which includes a serine/threonine protein kinase domain and (b) two regulatory subunits, named β (β1 and β2 variants) and γ (γ1, γ2, γ3). These different subunits are encoded by different genes.[1,4,10,13,14] The activation of AMPK by phosphorylation of Thr172 in the α subunit is a process that can be allosterically regulated by AMP (but not ADP)[15] and mediated by several upstream kinases, such as the liver kinase B1 (LKB1),[16,17] the scaffold protein mouse protein-25 (MO25), the pseudokinase STRAD[18-20] and calmodulin-dependent kinase kinases (CaMKKs), especially CaMKKβ.[21-23] AMP and ADP can induce phosphorylation of the α subunit Thr172 by LKB1 and CaMKKβ.[15-17,24] AMP and ADP also have the capacity to inhibit Thr172 dephosphorylation mediated by protein phosphatases, such as protein phosphatase 2C alpha (PP2Cα); with AMP inducing a 10-fold more powerful effect than ADP, both AMP and ADP being antagonized by ATP.[14,25,26] Ca^{2+}- and AMP-dependent pathways are fully independent. Thus, an increase in Ca^{2+} leads to the stimulation of CaMKKβ, increasing Thr172 phosphorylation and consequently activating AMPK.[27] Finally, a mechanism regulating AMPK in an AMP-independent manner through phosphorylation/dephosphorylation processes has been suggested. When associated with the β subunit of AMPK, the cell-death-inducing like-effector A (CIDEA) induces a degradation of AMPK through ubiquitination, reducing its activity.[28] AMPK structure and regulation will not be discussed in detail here but were deeply reviewed elsewhere.[1,4,10,29-31]

Different stimuli can induce AMPK activation: (a) a decrease in intracellular energy levels, such as hypoglycaemia and hypoxia, or (b) an increase in ATP consumption, such as food deprivation or muscle contraction.[1-4,30,31] Changes in the adenine nucleotides' ratio, induce AMPK phosphorylation subsequently leading to an inhibition of ATP-consuming processes, as fatty acid synthesis, and to a stimulation of catabolic processes, like fatty acid oxidation. Thus, the main effect of an activation of AMPK is to generate ATP and re-establish AMP:ATP and ADP:ATP ratios, in order to maintain a cellular energy homoeostasis.[1-4,30,31] In this sense, catabolic processes including autophagy (mitophagy) and mitochondrial biogenesis are switched on.[32-35] In the same way, anabolic processes such as the biosynthesis of lipids, proteins, carbohydrates and ribosomal RNAs, will be turned off by AMPK when the intracellular energetic levels are reduced.[1-4,30,31]

3 | HYPOTHALAMIC AMPK AND FOOD INTAKE

David Carling and *Caroline Small* research groups demonstrated for the first time that hypothalamic AMPK was implicated in the modulation of energy balance, with an important role in the regulation of feeding.[36] They showed that key hormones implicated in the control of food intake, such as leptin and ghrelin, could modulate AMPK at hypothalamic level, regulating appetite.[36] Similar work realized by *Barbara Kahn's* group established that AMPK was highly expressed in the different hypothalamic nuclei, such as the arcuate (ARC), dorsomedial (DMH), paraventricular (PVH) and ventromedial (VMH) as well as in the lateral hypothalamic area (LHA).[37] Notably, they have also determined that modulation of hypothalamic AMPK pathway could induce some adaptive change in the physiological regulation of feeding.[37] Thus, while AMPK activity is increased under fasting in numerous hypothalamic regions, it was shown to be inhibited when submitted to refeeding conditions.[36-38] Moreover, at the whole-body level, an increased activity of AMPK at a hypothalamic level induced an increase in food intake, consequently leading to a body weight gain, while its inhibition induced hypophagia associated with weight loss.[37]

In the same line with the previous physiological observations, the use of genetic models has confirmed a major role for hypothalamic AMPK in the regulation of food intake. Firstly, it was demonstrated that an inhibition of hypothalamic AMPK using dominant negative isoforms of AMPKα (AMPKα-DN) induced a decrease in the orexigenic neuropeptides agouti-related peptide (AgRP) and neuropeptide Y (NPY) mRNA expression in the ARC. Conversely, an over-expression of

constitutively active isoforms of AMPKα (AMPKα-CA) induced an increase in the expression of AgRP and NPY in the ARC, as well as in the expression of melanin-concentrating hormone (MCH) in the LHA.[37] Moreover, it has been reported that AMPK could regulate NPY and proopiomelanocortin (POMC) expression through a modulation of autophagy.[39] These observations suggest that AMPK could exert its modulation on feeding through hypothalamic nucleus-specific effects. This specificity was confirmed using genetically modified mice specifically ablated in the catalytic subunit of AMPKα2 in POMC or AgRP neurons of the ARC. Interestingly, the two models exhibited different phenotypes: while POMC AMPKα2 KO mice developed obesity due to hyperphagia, AgRP AMPKα2 KO mice developed an age-dependent lean phenotype.[40] Current evidence has also shown that AMPK in the paraventricular nucleus of the hypothalamus (PVH) modulates dietary preference for carbohydrate over fat,[41] expanding the role of hypothalamic AMPK on feeding control. However, despite the undeniable role of AMPK in the modulation of feeding, several recent studies might indicate that the effects of hypothalamic AMPK observed on body weight changes are more likely associated with an altered energy expenditure than to a modification of food intake.[42-52]

Remarkably, most of the actions of AMPK within the hypothalamus are elicited by different hormones. It is well-known that both orexigenic and anorexigenic hormones modulate hypothalamic AMPK to regulate appetite. The most spread and accepted theory is that anorexigenic factors inhibit hypothalamic AMPK while orexigenic ones activate it.[42-50] For example, the vast majority of physiological feeding inhibitors such as leptin,[36,37,53] glucagon-like peptide-1 (GLP-1),[48,54] oestradiol (E2),[47,55] insulin[37,56] and ciliary neurotrophic factor (CNTF)[57] are all described to inhibit hypothalamic AMPK. In contrast, the activation of hypothalamic AMPK was demonstrated to be induced by orexigenic signals such as ghrelin,[36,38,58-61] adiponectin,[62,63] glucocorticoids,[64] cannabinoids[58,65] and AgRP.[37] Nevertheless, resistin (RSTN), despite its anorectic effect, stimulates hypothalamic AMPK.[66] Hypothalamic AMPK being a key modulator of food intake opened new insights into pharmacological treatment of obesity, as for example, melanocortin receptor agonists (including melanotan II; MTII) or even nicotine, two well-known feeding-reducing compounds that exert their actions through the inhibition of hypothalamic AMPK.[37,46,67] Conversely, anti-psychotic drugs (APDs), such as olanzapine, well-known for their orexigenic properties, stimulate hypothalamic AMPK activity.[68-72] Taken all together, these data suggest that central AMPK could be a potential target for the treatment of obesity, an approach that it is strengthened by the effects of AMPK on energy expenditure (later discussed).[42-52]

4 | HYPOTHALAMIC AMPK AND THERMOGENESIS

Besides its role in the feeding behaviour regulation, the hypothalamus also plays an important role in the modulation of brown adipose tissue (BAT) thermogenesis activity through its action on the sympathetic nervous system (SNS). BAT is stimulated by increased firing of sympathetic neurons, leading to the release of noradrenaline and to the final activation of β3-adrenergic receptors (β3-AR).[73-77] At a central level, the VMH was the first hypothalamic nuclei playing a crucial role in BAT thermogenic activity to be described.[78] The VMH is connected to other brainstem regions implicated in the BAT activity modulation, such as the raphe pallidus (RPa) and the inferior olive (IO), two regions involved in the sympathetic activation of BAT.[73-76]

Numerous recent studies have described that hypothalamic AMPK had a major role in the modulation of BAT thermogenesis through its regulatory action on the SNS. We were able to determine through the study of the central effects of thyroid hormones (THs) on energy homeostasis that a VMH-specific injection of 3,3′,5-triiodothyronine (T3) was able to induce an increase in BAT thermogenic activity associated with a decrease in AMPKα phosphorylation levels in the VMH; importantly, that action run in parallel with an increase in the sympathetic firing on brown fat.[43,51,79-81] Interestingly, adenoviruses harbouring AMPKα-CA isoforms administered specifically in the VMH reduced BAT thermogenic activity, preventing the central T3 induced body weight loss in a feeding-independent manner.[43,51,81,82] Current data from our group have also demonstrated that AMPK inhibition orchestrates a coordinate response to central T3 by activating at the same time: (a) BAT thermogenesis and the browning of WAT, through SNS, on the one side and (b) the parasympathetic nervous system (PSNS)-dependent hepatic lipogenesis on the other side.[81,83] The molecular underpinnings of this effect depend on ceramide-induced endoplasmic reticulum (ER) stress [83,84] and activation of c-Jun N-terminal kinase 1 (JNK1) in the VMH, respectively.[81]

The physiological relevance of these findings is given by the fact that this integrative mechanism is not limited to THs. Indeed, it was demonstrated that the central action of E2, through its binding to oestrogen receptor alpha (ERα), decreased AMPK activity selectively in the VMH, inducing an increase in BAT thermogenic capacity through the SNS in a feeding-independent manner.[47,85-88] Moreover, an adenovirus-mediated activation of AMPK in the VMH (but not in the ARC) prevented the increase in BAT thermogenesis activity and body weight loss associated induced by centrally administered E2.[47] Interestingly, variations in E2 levels during the oestrous cycle and pregnancy were also

described to modulate the AMPK pathway, supporting also its physiological importance.[47,85] Recent studies have demonstrated that bone morphogenetic protein 8B (BMP8B) acts centrally on the modulation of BAT thermogenic activity depending on the activation status of AMPK in the VMH. Notably, the specific administration of AMPKα-CA isoforms in the VMH prevented the BMP8B-induced thermogenesis.[45,52] Similar results were obtained using pharmacologic antagonists or genetic deletion of orexin (OX) in the LHA.[52] Although the implication of OX neurons in the mediation of THs and/or E2 effects is currently unknown, the similitude in thermogenic responses observed and the known neuroanatomical connections suggest a possible participation, a hypothesis that will need to be confirmed. All together, these results highlight that the hormonal modulation of the VMH AMPK-(LHA OX)-SNS-BAT axis is an important element of energy balance.[49,50]

The demonstration that virogenetic manipulation of AMPKα1 in the VMH activated BAT thermogenesis and browning[43,45-48,52,81,82] prompted us to identify the specific VMH neuronal population of neurons mediating those effects. To this end, we generated a steroidogenic factor 1 (SF1)-specific AMPKα1 null mouse line by crossing floxed AMPKα1 mice with SF1 Cre mice, which expressed Cre recombinase under SF1 promoter hence targeting this subset of neurons of the VMH. SF1 AMPKα1 KO mice displayed feeding-independent reduced body weight and adiposity associated with increased energy expenditure, higher BAT activity and thermogenesis. Notably, those effects depended on SNS activation and were blocked by β3-AR antagonism.[81] Overall, this evidence demonstrates that AMPK activity within the VMH, and more precisely in SF1 neurons, is a physiological and canonical mechanism regulating BAT thermogenesis through modulation of sympathetic tone.[49,81,89,90] Thus, in line with these findings, the AMPK pathway within the VMH may appear as an attractive target in the treatment of obesity. As an example, subcutaneously administered nicotine induces BAT thermogenesis and associated weight loss through a modulation of AMPK in the VMH.[46,67] Moreover, liraglutide, a GLP-1 agonist currently used clinically for the treatment of type 2 diabetes (T2D), modulates AMPK specifically in the VMH, leading to an increase in BAT thermogenic activity associated to a browning of WAT; all this contributing to a significant weight loss.[48]

5 | AMPK AS A TARGET FOR METABOLIC DISORDERS

AMPK has become a potential therapeutic target in metabolic diseases involving impaired eating behaviours, including obesity, T2D and some lipodystrophies. AICAR was one of the first described activators of AMPK.[91] However, despite its effects on the improvement of glucose tolerance and on the reduction in circulating triglycerides (TG) and free fatty acids (FFA),[92] AICAR poor bioavailability and short half-life limit its use in human clinical trials.[93] Other AMPK activators, such as 991 and A-769662, have also been described to induce decreases in blood glucose and lipid levels.[94] However, as for AICAR, their therapeutic potential is impaired due to their effects on cell cycle progression limiting their therapeutic use.[95]

Decrease in ectopic lipid accumulation and improvement in insulin sensitivity can be a consequence of (a) increased glucose uptake by skeletal muscle[34,96] or of (b) an inhibition of hepatic glucose production.[97] Nowadays, various AMPK activators are prescribed to restore abnormally high glucose levels in T2D. Metformin, a synthetic biguanide, stimulates AMPK in an indirect manner through the inhibition of the mitochondrial respiratory chain.[98,99] It was described that metformin induced a decrease of 2% of haemoglobin HbA1c levels in T2D patients, with very few associated side effects. Interestingly, metformin also reduces the risk of cardiovascular disease[100] and various types of cancer.[101] Metformin main effect—the inhibition of hepatic glucose production[102]—was described to be dependent of LKB1 pathway. Therefore, as LKB1-null animals do not exhibit reduced blood levels of glucose,[19,103] it suggests that metformin action may be mediated by an indirect activation of AMPK. Recently, it was described that metformin could exert its effects in the liver in an AMPK-independent manner.[104] Oppositely, animals exhibiting ACC mutations were described to be refractory to the metformin-mediated improvement of lipid and glucose levels.[105] Other compounds such as the thiazolidinediones, (in particular rosiglitazone and pioglitazone) can also induce a drastic increase in AMP levels in skeletal muscles leading to a fast activation of AMPK.[106] These drugs may also have potential effects on the indirect activation of AMPK, acting on the peroxisome proliferator-activated receptor-gamma (PPARγ), which in turn induces adiponectin release.[107] Other drugs, such as liraglutide or the synthetic form of exendin-4, act as GLP-1 agonists leading to an increased insulin sensitivity.[108] However, liraglutide has been described to act in an opposite way on AMPK. While increasing AMPK phosphorylation in different tissues: (a) endothelium (decreasing inflammation),[109,110] (b) heart,[111] (c) liver and muscle (improving insulin sensitivity),[112,113] and (d) WAT,[114] liraglutide reduces AMPK phosphorylation in pancreatic beta cells (inducing cell proliferation)[115] and in the hypothalamus.[48] Liraglutide has also been described to increase BAT thermogenic capacity.[48] Moreover, Exendin enhances hepatic AMPK phosphorylation, leading to an improvement of steatosis.[116]

Besides these synthetic compounds, some natural molecules have also been described to improve metabolic

conditions through AMPK activation. For instance, resveratrol, a natural phenol located in the membrane of red grapes, is able to increase the levels of intracellular Ca2+, which will activate CaMKKβ,[117] leading to the indirect activation of AMPK and subsequently to an increase in muscle glucose uptake.[118] Resveratrol has also been described to decrease hepatic lipid accumulation, a mechanism that is dependent of AMPK as the effects are abolished when AMPK is genetically blocked.[119] Quercetin, one of the most abundant polyphenol encountered mostly in plants, has been described to possess metabolically protective roles. It has been shown that quercetin could have an anti-adipogenic action through the activation of AMPK and its direct substrate ACC.[120] Moreover, some studies suggest that quercetin could improve glucose metabolism in liver and skeletal muscle through an insulin-independent mechanism involving AMPK activation.[121] Quercetin has also been described to decrease the cholesterol-induced neurotoxicity.[122,123] Other natural compounds, such as barberine and rooibos, were described to induce beneficial effects on glucose homeostasis and cholesterol levels; these effects being mediated by an activation of AMPK in liver,[124,125] muscle [126] and adipose tissue.[125,127]

It is well-known that metabolic disorders, such as obesity, T2D and insulin resistance, are risk factors increasing cancer development.[128] As AMPK is implicated in cellular metabolism—its activation blocks the cell cycle through an inhibition of anabolism—it is relevant to consider that AMPK could limit tumour development. As tumours with specific AMPK mutations are rare in humans, it is more likely to assess those tumorigenic processes will rather be a consequence of defective upstream and/or downstream actors of AMPK, rather than AMPK directly. In this sense, the inactivation of LKB1 activates mTORC1, leading to cell proliferation,[19,103] while mutations in LKB1 will inactivate AMPK, leading to Peutz-Jeghers syndrome, a cancer risk factor.[19] Recent studies were able to show that an inactivation of AMPK could stimulate the aerobic glycolysis leading to oncogenes activation and tumour suppressors inhibition.[129] Regarding this, targeting AMPK activation has been suggested as a possible therapeutic approach in cancer. In this sense, the AMPK activator metformin was used to induce a decrease of tumour size in mice.[101] Moreover, high levels of pACC have been found in prostate cancer cells, suggesting an important role of AMPK in this type of tumours.[130] It is known that AMPK is in an active state when energy levels are low; therefore, it is logical to advance that a continued AMPK activation might be crucial for cancer cell survival. However, to confirm AMPK role in tumorigenic processes, further investigations are essential. In this sense, recent studies demonstrated that AMPK was implicated in the regulation of glycolysis and cell survival in response to mitophagy during mitotic arrest.[12]

6 | HYPOTHALAMIC AMPK AS A TARGET FOR OBESITY

Obesity and its associated metabolic complications, such as cancer, cardiovascular diseases and T2D, cause numerous deaths per year worldwide. However, obesity is also considered as the most preventable epidemic.[108,131-133] However, despite numerous important investments in education and public engagement, government-led strategies are relatively unsuccessful. As stated in the *World Health Organization* (WHO)'s latest report, 13% of adults are considered obese worldwide. In healthful subjects, maintaining a standard bodyweight is a matter of lifestyle. Nevertheless, a precise comprehension of how the organism regulates energy balance is essential to develop new therapeutic strategies.

Numerous recent studies have highlighted an undeniably key role of hypothalamic AMPK in the modulation of the 2 components of the energy balance equation, that is feeding and energy expenditure[42,44,49,50,89,90] (Figure 1). As already mentioned above, the antidiabetic drug, metformin exerts its function through an activation of AMPK in peripheral organs (for an extensive review see[99]). However, it is well-known that the metabolism associated regulation of AMPK between periphery[134] and the brain[37,38,44,61,134] is differential. Therefore, a central activation of AMPK would not be a suitable strategy in obesity treatment, as it will increase feeding while decreasing BAT thermogenesis. Inhibiting AMPK in peripheral tissues would worsen insulin resistance and diabetes, raising the importance of the specificity of the treatment. In this sense, the best strategy would be to target the hypothalamic AMPK in a specific manner. However, the complexity of the anatomical and structural network renders the task highly complex. Drawing from what was carried out in other diseases, the use of nanoparticles or exosomes[135] might be an innovative strategy to target specifically hypothalamic populations, for example AMPK SF1 neurons in the VMH. Another relevant strategy would be optogenetic neuromodulations of hypothalamic AMPK neurons.[136] However, the use of optogenetics raises some ethical and technical problems in human interventions limiting this approach. One more realistic approach would be the use of chimeric proteins (targeting peptides associated with effective molecules or steroid hormones) to increase the specificity of action and limiting side effects.[132,133,137-139] In these lines of findings, chimeras containing GLP-1 plus an oestrogen,[137] or glucagon plus TH,[139] could increase the specificity of the AMPK neuronal targeting in the VMH. However, although the specificity will be increased using these strategies, other neuronal populations would still be affected[140] limiting efficiency and likely causing side effects.
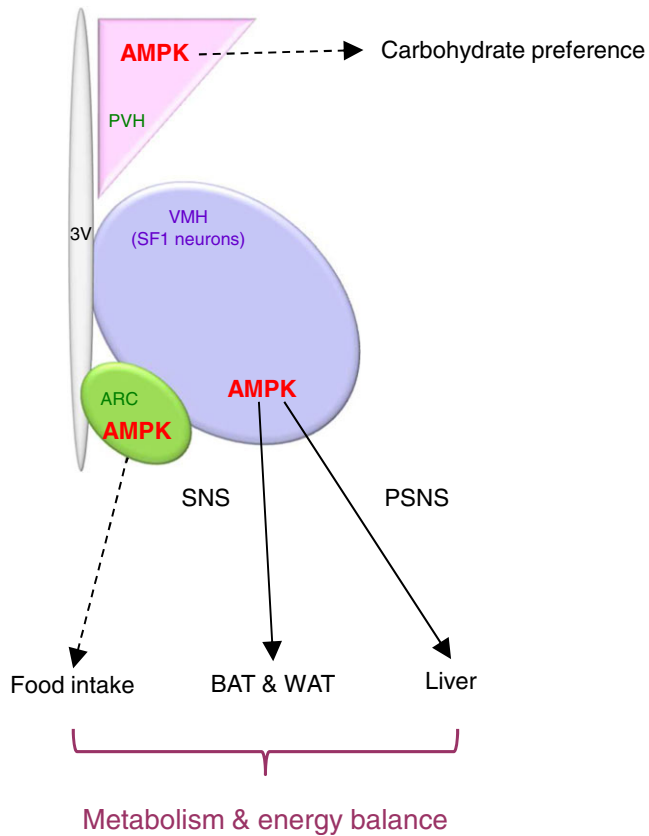
FIGURE 1 Hypothalamic AMPK is a canonical regulator of energy balance. AMP-activated protein kinase (AMPK) acts in the hypothalamus to modulate whole-body energy homoeostasis and body weight. AMPK senses several nutritional and hormonal stimuli to regulate food intake, peripheral metabolism, brown adipose tissue (BAT) thermogenesis, and browning of white adipose tissue (WAT). The actions of hypothalamic AMPK on peripheral tissues/organs, such as BAT, WAT and liver, are mediated by specific regulation of the sympathetic (SNS) and parasympathetic nervous systems (PSNS), respectively. 3V: third ventricle; ARC: arcuate nucleus of the hypothalamus; PVH: paraventricular nucleus of the hypothalamus; VMH: ventromedial nucleus of the hypothalamus

All over, these exposed limitations raise some questions about the translatability of these strategies into human clinical trials. Albeit the targeting of hypothalamic AMPK can be achieved quite precisely nowadays, other issues emerge. In my point of view, the main concern is the one of some long-term consequences of targeting AMPK within the brain. It has been well-described that AMPK has a central implication in numerous functions, such as lipid and glucose metabolism; therefore, how hypothalamic neurons would behave in answer to sustained AMPK inhibition? Would this affect their survival rate? In this context, recently published studies have demonstrated that impaired lipid metabolism in neurons could induce lipotoxicity, endoplasmic reticulum stress as well as leptin and insulin resistance,[83,84,141-145] all these participating in the development of major side effects. The concern of interconnection

with other hypothalamic-mediated physiological processes, such as regulation of endocrine axes, is also raised. Would AMPK modulation impact them on the long term?.[60] To address all these interrogation marks, a considerable work would be needed to understand deeply and fully the neuronal and molecular AMPK pathways and all its interconnected mechanisms, a fascinating endeavour for the years to come.

CONFLICT OF INTEREST

The author declares no competing (financial, personal or professional) interests.

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