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Hypothalamic Akt/PKB signaling in regulation of food intake

Dong-Hoon Kim[1], Stephen C. Woods[2], Randy J. Seeley[3]

[1]Department of Pharmacology, Korea University College of Medicine, Seoul, Republic of Korea, [2]Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati, Cincinnati, Ohio, USA, [3]Department of Internal Medicine, University of Cincinnati, Cincinnati, Ohio, USA

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1. ABSTRACT

Recent tremendous advances in our understanding of the regulation of food intake are expected to contribute to the treatment of obesity in the near future. The hypothalamus is a center for regulation of food intake and NPY/AgRP and POMC neurons are key regulators of food intake in the arcuate nucleus. The level of energy in the body is monitored by energy sensors in the hypothalamus. A variety of signals originating from peripheral organs to sense the status of energy converge on the hypothalamus and diverse neurons in the hypothalamus are involved in determining the output of signal to regulate food intake. Therefore, it is important to understand the signals and energy sensors in the hypothalamus. In this review, we describe the potential role of Akt/PKB signaling as an energy sensor that regulates food intake.

2. INTRODUCTION

Obesity is a state of an individual having excess body fat defined as body mass index equal to or more than 30 by the World Health Organization (1). The prevalence of obesity is rapidly growing and it has become a major health problem in undeveloped as well as developed countries. The imbalance induced by increased food intake and decreased physical activity causes severe obesity (2, 3). That said, body weight is normally quite precisely maintained by a balance between energy intake and energy expenditure (4). Our body has an integrative system to accurately maintain energy homeostasis by communicating via a variety of signals. The brain is a key part of controlling each arm of energy balance by sensing numerous inputs from central or peripheral organs such as gut, adipose tissue, pancreas, liver and central nervous

system. The signals can be hormonal, neuronal, or nutritional (5, 6). Neurons in specific regions of the brain respond to the inputs by sensing and integrating them. Many intracellular signaling molecules in neurons are involved in this process, with each having unique responsiveness to specific signals. Through this interaction, the neurons involved in energy homeostasis determine the output for maintaining whole energy balance. The output displays as meal initiation, termination, or a change of energy expenditure (6).

In this review, we describe the regulation of energy balance in the hypothalamus and related neuroendocrine systems. We focus on signaling molecules involved in the process of fuel sensing and especially the role of Akt/protein kinase B (Akt/PKB) in the hypothalamus.

3. CNS REGULATION OF ENERGY BALANCE

3.1. CNS regions regulating food intake

Food intake is a complex voluntary behavior involved in many neural circuits of the brain. Recent advances in understanding the neurobiology of energy balance allow us to categorize them into three parts (5, 6). First is the circuit regarding satiation signals, which arise during meals and control meal size following the pattern of cholecystokinin (CCK). The brain regions receiving signals from the liver- and gut-brain axis for satiation signals include the area postrema (AP) and the nucleus tractus solitarius (NTS) in the hindbrain. Second is related to adiposity signals such as leptin and insulin, which are released respectively from the adipose tissue and pancreas in proportion to the amount of body fat and transported in blood to neurons in the hypothalamus, hindbrain and other regions. Last are neural circuits related to food reward and pleasure including the prefrontal cortex (PFC), nucleus accumbens (NAc), and mesolimbic dopamine system. Although each of these is categorized and dominantly acts on its specific function and region of the brain, they are coordinated and integrated with other signals in the various brain regions (5-7).

The hypothalamus is a critical player for regulation of food intake. Various regions in the hypothalamus such as the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial hypothalamus (VMH), perifornical area (PFA) and the lateral hypothalamic area (LHA) are involved in the regulation of food intake (8). Circulating leptin and insulin reach neurons in the ARC and act on at least two neuronal populations, neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons and proopiomelanocortin (POMC) neurons. These in turn signal differentially to second-order neurons expressing thyrotropin-releasing hormone, corticotropin-releasing hormone, arginine vasopressin and oxytocin in the PVN, and melanin-concentrating hormone (MCH) and orexins in the LHA and PFA (6, 8).

In addition to this hypothalamic system, the hindbrain is also an important area for the regulation of food intake. Leptin receptors are expressed there and leptin can directly affect neurons within the NTS, where signals via vagal afferents or hormones released from the intestines or other peripheral tissues converge. CCK generated in the intestines in response to food consumption plays a role in meal termination via interaction with neurons within the NTS. In this region, the signal brought by CCK can be coordinated or integrated with the action of leptin to influence ongoing meals (9, 10).

Food intake is regulated by not only the integrated action of neurocircuits on signals from peripheral organs but also via the involvement of cognitive, visual, and taste inputs, among many others, in the brain. The neurocircuits related to food reward, such as those in the NAC, amygdala, VTA, globus pallidus in the striatum and the cortical area, are intimately connected to the LHA, where food intake is stimulated and is controlled by tonic inhibition of signals from adjacent hypothalamic areas (5). Dopamine is important for augmentation of 'wanting', which increases the incentive salience of food, and mu-opioid receptor activity is important for 'liking', which includes objective affective reactions to pleasurable stimuli are important components to deliver the signals in the reward neurocircuits, especially from the VTA to NAC within the striatum and other regions in the brain (7, 11, 12)

3.2. Neuroendocrine sensing of adiposity signals in the ARC

Circulating leptin and insulin increase or decrease depending on the amount of stored body fat. Both can cross the blood-brain barrier and act on their receptors in specific regions of the brain, especially the ARC. Both hormones act on two distinctive neuronal populations, POMC neurons and NPY/AgRP neurons in the ARC, which are important components of catabolic and anabolic effector pathways of energy balance (8, 13).

POMC neurons in the ARC, in response to increased action of adiposity signals, increase synthesis of the large precursor polypeptide, POMC, which is cleaved into a number of biologically active peptides via multiple post-translational processing steps. Among the cleaved products, alpha-MSH plays a critical role in catabolic action of energy balance. Central administration of exogenous alpha-MSH or its synthetic analogues decreases food intake and body weight mainly via melanocortin-4 (MC4) receptors in the hypothalamus (14-19) while SHU9119, a melanocortin antagonist at the MC3 and MC4 receptor and HSO14, a selective antagonist at the MC4 receptor increases food intake (15, 20-23). The phenotype of MC4 receptor-deficient mice includes increased food intake and body weight and reveals the crucial role of melanocortins in the tonic inhibition of food intake (24).

NPY/AgRP neurons in the ARC produce both NPY and AgRP (25, 26), which increase food intake and body weight (27-29) in opposite to the action of POMC neurons. The expression of NPY and AgRP is increased in fasting and when leptin is low (25, 30, 31). However, rapid ablation of NPY/AgRP neurons induces severe anorexia in adult mice (32, 33). In contrast, genetic deletion of NPY or of AgRP (34, 35), rapid ablation of NPY/AgRP neurons

during the neonatal period (32), or gradual ablation (36-38), induces only mild effects on energy balance. This suggests that compensatory mechanisms for chronic deletion of NPY/AgRP neurons occur. Therefore, NPY/AgRP neurons and POMC neurons in the ARC are crucial players for energy balance by integrating adiposity signals informing the state of the stored body fat.

3.3. Nutrient sensing of metabolic substrates in the ARC

Metabolic substrates such as glucose and fatty acids also play a role as signals in carrying information about the energy status to the neurons in the ARC. Jean Mayer proposed the 'glucostatic theory', postulating that the glucoreceptors in the hypothalamus can monitor the level of blood glucose in order to regulate food intake (39, 40). This hypothesis is supported by the findings that central administration of 2-deoxy-D-glucose (2-DG), a glucose analog that inhibits glycolysis and causes intracellular glucopenia, increases food intake. In addition, central 2-DG is more efficacious at stimulating food intake than peripheral 2-DG (41), and local administration of 2-DG into hypothalamus or hindbrain increases neuronal activity in these areas (42-44). Ambient glucose levels are rapidly sensed in the hypothalamus as well as peripheral tissues depending on the situation and this fluctuating glucose level is monitored by "glucosensing" neurons located there. The glucosensing neurons can be subdivided into glucose-excited neurons and glucose-inhibited (43, 45, 46). Both neurons reciprocally respond to the rapid changes of ambient glucose levels to regulate food intake.

Although the mechanism by which the glucose sensing neurons sense the change of glucose levels is uncertain, ATP-sensitive potassium channels that exist in neurons but not in glia, are thought to be mainly involved in this process (47). The K_{ATP} channel is composed of two subunits, Kir6.2 and SUR1. Similar to pancreatic beta cells secreting insulin in response to glucose (48), when glucose is oxidized to generate ATP in neurons, the increased intracellular ATP:ADP ratio increases intracellular K^+ level via inactivating or closing inwardly-rectifying potassium channels after ATP directly binds to the Kir6.2 subunit. This causes depolarization of the neurons and increases the firing rate by increasing Ca^{2+} influx (44, 49). The 'glucose-excited' neurons, like pancreatic beta cells, play an important role in regulation of blood glucose levels in response to ambient glucose. The failure to close K_{ATP} channels selectively in POMC neurons by overexpression of the mutant Kir6.2 subunit impairs glucose tolerance (50). Pharmacologic inhibition of the K_{ATP} channels or a genetic deletion of the SUR1 subunit of the K_{ATP} channels suppresses the inhibitory action of insulin on hepatic gluconeogenesis in rats or mice, indicating the direct link between the hypothalamus and liver (51).

There are also several reports supporting the role of K_{ATP} channels in the regulation of food intake. Leptin hyperpolarizes and inhibits hypothalamic neurons depending on phosphatidylinositol-3-OH kinase (PI3K) signaling by activating K_{ATP} channels (52, 53). 2-DG induced increases in food intake are blunted in the Kir6.2 mutant mice, and this regulation of food intake is independent of leptin and NPY (54). However, it has been also found that the phosphatidylinositol-(3,4,5)-triphosphate phosphatase, Pten deficiency in POMC neurons, which enhances phosphatidylinositol-3,4,5-trisphosphate (PIP3) signaling, causes diet-induced obesity and hyperphagia in mice by activation of the K_{ATP} channels. In these mice, leptin is not able to increase the neuronal activity of POMC neurons, which is restored by PI3K inhibitors and by the K_{ATP} blocker, tolbutamide (55). Therefore, these studies support the involvement of K_{ATP} channels in regulation of food intake.

Although it is not yet clear whether neurons use lactate formed by metabolizing glucose or from the anaerobic glycolysis in astrocytes (56), lactate followed by pyruvate is essential for regulation of glucose via the K_{ATP} channels in the hypothalamus (57). Central administration of pyruvate suppresses the increased food intake induced by 2-DG via presumably AMPK-activated protein kinase (AMPK) in AgRP neurons triggered by a change of ATP levels (58). Central administration of lactate or pyruvate decreases food intake and body weight, and the change of food intake induced by circulating lactate requires hypothalamic lactate sensing (59).

In spite of a range of evidence about the role of glucose or its metabolism in regulation of food intake, the 'glucostatic hypothesis' has limitations. Eating occurs independent of the fluctuating plasma glucose levels in a normal situation. Most meals occur when blood glucose is normal or high. Animals eat while glucose is infused (60). Therefore, there may be a limited role of glucose in normal circumstances, but it may be most important under extreme situations of low glucose.

In addition to glucose, fatty acids play a role to potentially impact fuel sensors in the brain. Central administration of oleic acid, a long-chain fatty acid, but not octanoic acid, a short-chain fatty acid, reduces food intake and body weight (61). Modulation of fatty acid synthesis and lipid oxidation also affect food intake although fatty acids cannot be used as a fuel in neurons. Formation of malonyl-CoA, the commitment step of fatty acid synthesis, is crucial for sensing fatty acids in the neurons of the hypothalamus, but not fatty acid synthesis per se because only inhibition of fatty acid synthase (FAS), a multifunctional polypeptide complex, decreases food intake while inhibition of the production of malonyl-CoA using acetyl-CoA carboxylase inhibitors, does not affect food intake (62). The suppression of carnitine palmitoyltransferase-1 in the hypothalamus, a key regulator of lipid oxidation by regulating the entry of long-chain fatty acid into mitochondria, sufficiently decreases food intake, and hypothalamic overexpression of malonyl-CoA decarboxylase (MCD) involved in degradation of malonyl-CoA decreases food intake (63, 64). Therefore, hypothalamic malonyl-CoA is an important player in feeding behavior and indicator of energy status (65) (Figure 1). However, this anorectic action of FAS inhibitor within the CNS does not solely depend on increased malonyl-CoA and subsequently long-chain fatty acids. The ample levels of glucose within the neurons and alternative fuel from glia

such as glutamine and lactate to alleviate the demand of neuronal glucose are crucial to determine the action of FAS inhibitor in food intake (66). The anorectic action of FAS inhibitors and long-chain fatty acid are mediated via a suppression of the fasting-induced increase of NPY/AgRP gene expression and decrease in POMC, however, repeated administration of FAS inhibitor blunts the change in hypothalamic gene expression (62, 67, 68). The hypothalamic FAS system is associated with two important metabolic sensors, AMPK and mammalian target of rapamycin (mTOR) in the regulation of food intake (69, 70).

4. HYPOTHALAMIC AKT/PKB SIGNALING IN THE REGULATION OF FOOD INTAKE

Numerous signals converge onto neurons in the hypothalamus and initiate a cascade of phosphorylation steps in the signaling pathway. Since the *ob* gene was cloned and its role in energy homeostasis was discovered (71, 72), numerous studies helped understand the action of leptin in regulation of food intake. Leptin exerts its effects via interaction with specific receptors located in distinct classes of neurons. Leptin initiates its action by binding to the leptin receptor (Lepr) in the hypothalamus. Various isoforms exist, as there is a short form of Lepr and a long form (LeprB). While several isoforms of the leptin receptor have been identified, the LeprB form that includes the long intracellular domain that has signaling capacity appears to mediate most of the biological effects of leptin (5,6). LeprB is predominantly expressed in hypothalamus, and mutation of LeprB in hypothalamus increases body weight and food intake as occurs in db/db mice (73). Both isoforms activate the Janus kinase (JAK2) and insulin receptor substrate (IRS) pathways, however, only LeprB can phosphorylate signal transducer and activator of transcription (STAT3). LeprB has a much more potent action in reducing food intake than the short-form of Lepr through the JAK2/STAT3 signaling pathway. When leptin binds to hypothalamic LeprB, JAK2 undergoes dimerization and phosphorylates STAT3 (74). pSTAT3 enters the nucleus and is involved in regulating gene transcription. The existence of pSTAT3 in hypothalamus is an important indicator for activation of leptin signaling (75). The STAT3 pathway was the first signaling mechanism associated with the leptin receptor (7). Neural-specific inactivation of STAT3 leads to hyperphagia and obesity in mice (8). In addition, disrupting the ability of the leptin receptor to activate the STAT3 pathway in mice leads to severe obesity and several other neuroendocrine abnormalities (9–11). More recently, other intracellular signaling mechanisms, including PI3K (12), AMPK (13), mTOR (14), have been shown to play an important role in the action of leptin on food intake.

In many aspects, hypothalamic pathways involved in leptin-induced anorexia overlap with those involved in insulin's intracellular actions. Hypothalamic PI3K, which is an important downstream mediator of leptin's actions, is also regulated by insulin via JAK2-medicated phosphorylation of IRS (76-78). Although the impact of PI3K signaling involved in leptin-induced anorexia is less potent than JAK/STAT3 signaling pathway, PI3K inhibitors in hypothalamus also block the anorectic action of leptin (76). In addition to this, the action of both leptin and insulin in the hypothalamus control the regulation of food intake by acting on other important signaling molecules such as forkhead transcriptional factor subfamily forkhead box O1 (FoxO1) (79), mTOR (80), suppressor of cytokine signaling (SOCS3) (81-83), protein tyrosine phosphatase 1B (PTP1B) (84) and inhibitor of kappa B kinase beta (IKK beta) (85).

PI3K is a key molecule for insulin and leptin-induced reduction of food intake (76, 86). When insulin binds to the insulin receptor (IR), IRS is phosphorylated on tyrosine residues. Activated IRS converts phosphatidylinositol-4,5-bisphosphate (PIP2) to PIP3, which further activates phosphoinositide dependent protein kinase-1 (PDK1). PDK1 initiates Akt/PKB enzymatic cascades by phosphorylation of Akt/PKB on Thr308. Activated Akt/PKB is involved in a variety of cellular functions, including growth, angiogenesis, proliferation, glucose uptake, metabolism and survival. These cellular processes are mediated by regulation of multiple substrates *such as FOXO, IKKβ, BAD, Casp9, AS160, eNOS, TSC2, PRAS40, p27, MDM2, and GSK3* (87). Among these substrates, FOXO1 and mTOR are key targets of Akt/PKB regarding regulation of food intake (79, 80).

Several signaling molecules close to the PI3K/PDK1/PKB signaling pathway involved in the regulation of food intake have been reported (76, 79, 80, 86, 88, 89). Upstream of this pathway, IRS2 and the IR play a role in regulating energy balance (88, 89). Neuron-specific insulin receptor knockout (NIRKO) mice have increased food intake and obesity, and mild insulin resistance with hypertriglyceridemia. The NIRKO mice also have higher leptin levels consistent with greater adiposity. The phenotype of NIRKO mice was more prominent in female mice. The neuronal disturbance of IR signaling induces an impaired reproductive system (88). The deletion of IRS-2, which is rapidly phosphorylated on tyrosine residues in response to insulin and insulin-like growth factor-1, also causes increased food intake and body weight in mice similar to NIRKO mice. The increased food intake occurs in spite of higher leptin levels without activation of hypothalamic STAT3 signaling, indicating the essential role of IRS2 in regulation of food intake mediated by leptin. It also supports that the hypothalamic leptin resistance is associated with insulin action in the nervous system (89). A study on the role of hypothalamic insulin in regulation of food intake using two PI3K inhibitors, wortmannin and LY294002, strongly supports the involvement of insulin signaling in phenotypic findings of the IRS2 and NIRKO knockout mice (86). In this study, systemic or intraventricular administration of insulin into the ventricle adjacent to the ARC increases the expression of PI3K in the ARC and the anorectic effect of insulin on food intake is reversed by the PI3K inhibitors. PIP3 and IRS2 are colocalized in ARC neurons indicating the preferential insulin-induced PI3K activity within cells containing IRS-2 (86). As mentioned earlier, leptin also reduces food intake via PI3K signaling (76). Both leptin

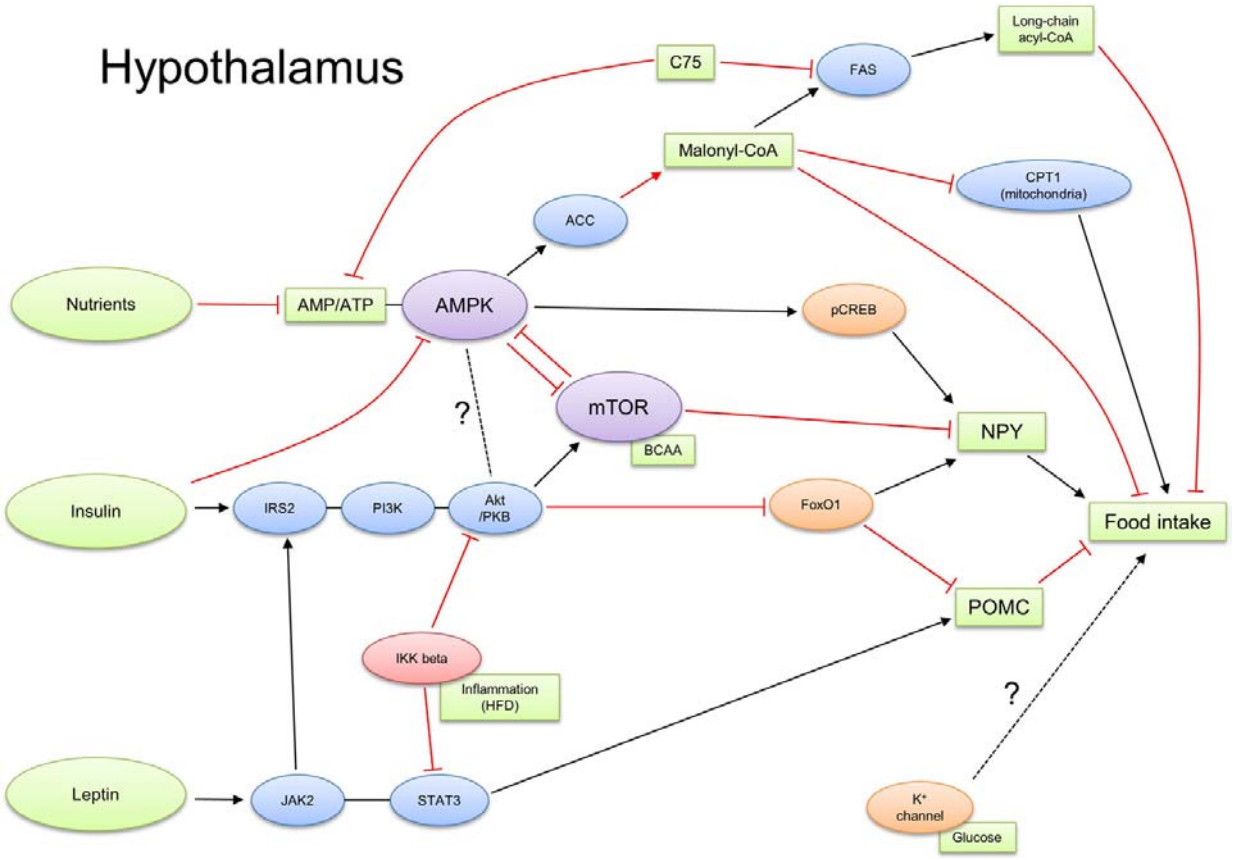

Figure 1. Convergent intracellular signaling cascades in the hypothalamus regarding food intake. Hormones and nutrients produce a change of food intake by modulating the activity of signaling molecules in the neurons within the hypothalamus. The intracellular signaling molecules participate in the process of food intake regulation by responding to a specific signal and also interacting with other signaling molecules. Leptin and insulin have a common signaling pathway to regulate food intake in the hypothalamus via IRS. AMPK and mTOR has a reciprocal action in food intake. AMPK-ACC-Malonyl-CoA axis is an important regulator of food intake. Each line represents the interaction between two signaling molecules. Black line indicates the positive regulation and red negative. BCAA means branched amino acid.

and insulin stimulate activity of PI3K in POMC neurons in parallel and in AgRP neurons in an opposite way. This leptin-mediated PI3K activation in POMC neurons does not require STAT3 activation (90). The acute but not chronic anorectic action of leptin is mediated via PI3K activity in POMC neurons. Mice with deleted function of PI3K in POMC neurons by reducing the level of p85 subunit show normal long-term energy homeostasis (91). Although acute leptin and insulin regulate PI3K signaling in POMC neurons, their populations within mediobasal hypothalamus are distinctive (92). The role of PI3K in energy balance is not limited to the ARC. Specific deletion of p100 alpha subunit of PI3K in steroidogenic factor 1 (SF1) neurons of the VMH increases body weight in mice by reducing increased energy expenditure (93). These reports suggest that hypothalamic insulin signaling pathway upstream of Akt/PKB signaling is an important regulator for food intake and associated with hypothalamic leptin signaling in regulation of food intake.

Downstream of PI3K signaling pathway, several key molecules play a role as a fuel sensor to regulate food intake. FoxO1, one substrate for Akt/PKB, is involved in regulation of energy balance in the hypothalamus (79, 94) (Figure 1). Activation of Akt/PKB inhibits action of FoxO1 via polyubiquitination-induced proteosomal degradation and nuclear excursion in vitro and in vivo (95, 96). Administration of insulin or leptin, which reduces food intake, suppresses hypothalamic expression of FoxO1 and PI3K inhibitor blocks the suppressive effect of insulin and leptin on FoxO1. Hypothalamic FoxO1 increases food intake and body weight by stimulating the transcriptional level of NPY/AgRP and suppressing POMC. FoxO1 directly stimulates promoter activity of NPY by binding to insulin responsive elements (IREs) and AgRP, but indirectly that of POMC through inhibition of STAT3 (79).

Although IKK beta/NF-kappa B may not be a direct target for Akt/PKB regarding regulation of food intake, constitutional activation of IKK beta in the mediobasal hypothalamus impairs the suppressive action of insulin in fasting-induced increases of food intake in mice and also inhibits the phosphorylation of Akt/PKB in the hypothalamus induced by ICV administration of insulin.

This was confirmed by immunohistochemistry staining of PIP3 in hypothalamus (85). High-fat diets containing saturated acyl-CoA cause hypothalamic insulin insensitivity via inflammation because of the fat, but not the excess calories (97). However, the occurrence of hypothalamic insulin resistance depends on the type of fatty acid. Oleic acid, a monounsaturated fatty acid, in the hypothalamus decreases food intake as well as hepatic glucose production (61). Palmitic acid causes hypothalamic insulin resistance via increased localization of PKC-theta to membranes in the hypothalamus (98). The inhibitory action of IKK beta/NF-kappa B in regulation of food intake also extends to hypothalamic leptin signaling. Hypothalamic activation of IKK beta blunts leptin-induced phosphorylation of STAT3 in the hypothalamus and reduces the suppressive action of ICV leptin in fasting-induced increase of food intake (85) (Figure 1).

Besides the involvement of IKK beta/NF-kappa B in leptin resistance, which is often defined as a state of decreased LeprB signaling induced by prolonged stimulation of LeprB with leptin, there have been several important advances in revealing its mechanism. Activated JAK2 stimulates phosphorylation of 3 tyrosine residues like Tyr^{985}, Tyr^{1077} and Tyr^{1138}. Each tyrosine residues has own downstream target to recruit. Tyr^{985} residue stimulates SHP2 or protein tyrosine phosphatase, non-receptor type 11 (PTPN11) and subsequently extracellular-signal regulated kinase (ERK) pathway. Phosphorylation of Tyr^{1107} residue recruits STAT5 signaling pathway and Tyr^{1138} residue STAT3 (99). A part of studies reveal that LeprB signaling itself has a negative feedback loop. Activation of STAT3 at the Tyr^{1138} residue increases the accumulation of SOCS3, which binds to Tyr^{985} residue decreasing LeprB signaling (83, 100, 101). Female mutant mice at the Tyr^{985} residue show decreased food intake, resistance to diet-induced obesity and hypersensitivity to leptin (102). Neuronal specific SOCS3 deficient mice are resistant to diet-induced obesity and show an enhancement of hypothalamic STAT3 responsiveness to leptin and POMC (100). In addition to SOCS, PTPB1 participates in leptin resistance via mediating the process of direct dephosphorylation of JAK2 (103, 104). Whole body PTP1B deficient mice have lower adiposity with increased energy expenditure and increased insulin sensitivity (105, 106). Brain PTPB1 contributes to the reduced body weight. Neuronal PTP1B deficient mice have lower adiposity and body weight on both chow and high fat diet while there is no change of body weight in muscle- and liver-specific PTP1B knockout mice and increased body weight in adipose-specific PTP1B deficient mice. Neuronal PTP1B deficient mice show reduced food intake as well as increased energy expenditure and activity, and are also hypersensitive to leptin (84). Recently, it is reported that POMC neuron-specific PTPB1 knockout mice have reduced adiposity, increased energy expenditure and leptin sensitivity while POMC neuron-specific SHP2 knockout mice show opposite phenotypes indicating that PTP1B and SHP2 in POMC neurons are reciprocal regulators of energy balance (107). Finally, endoplasmic reticulum (ER) stress and low-grade inflammation induced by obesity plays an important role in development of leptin resistance. Increased ER stress and the unfolded protein response (UPR) signaling pathway causes leptin resistance in the hypothalamus of obese mice and pharmacologic administration of chemical chaperones such as 4-phenyl butyric acid (PBA) and tauroursodeoxycholic acid (TUDCA) to reduce ER stress improves leptin sensitivity. Genetic depletion of XBP1 in the CNS of mice, which is a key regulator of ER folding capacity, causes severe leptin resistance and aggravation of diet-induced obesity (108). MyD88 deficiency in the CNS, which is essential for TLRs signaling and the induction of proinflammatory cytokines, protects from diet-induced obesity and leptin resistance (109).

4.1. Mammalian target of rapamycin (mTOR)

One of the main targets for Akt/PKB is mTOR, which is a member of the phosphatidylinositol kinase-related protein kinase family. mTOR is also called as *FRAP, RAFT, RAPT, or SEP* and is critically involved in regulation of growth and development by stimulating protein synthesis or inhibiting autophagy (87, 110). Originally, it was known to exist in an evolutionarily conserved complex, which is rapamycin-sensitive (mTOR complex 1) by binding to two proteins such as regulatory-associated protein of mTOR (raptor) and G protein β-subunit-like protein (GβL). The mTOR complex 1 phosphorylates S6K1 at multiple residues (111-113). mTOR also forms another signaling complex, which is rapamycin-resistant (mTOR complex 2), with GbetaL and AVO3, or rapamycin-insensitive companion of mTOR (rictor). The mTOR complex 2 mediates Akt/ PKB phosphorylation in conjunction with PDK1 phosphorylation at the upstream of S6K1 (114). After insulin activates Akt/PKB through IRS/PI3K/PKB signaling, Akt/PKB phosphorylates Tuberous Sclerosis Complex protein 2 (TSC2), which inactivates TSC1/2 and increase GTP-bound Ras homolog enriched in brain (Rheb). Activated TSC1/2 through GTPase-containing domain converts GTP-bound Rheb into the GDP-bound Rheb. The increased GTP-bound Rheb directly activates mTOR complex 1 inducing phosphorylation of S6K1. S6K exists as two isoforms such as S6K1 and S6K2. S6K1 also has long or short isoforms. S6K1 increases protein synthesis via S6 ribosomal protein, EIF4E binding protein, end eukaryotic elongation factor 2 kinase (112, 114). S6K1 knockout mice are resistant to diet-induced obesity and insulin-sensitive (115).

Cota and colleagues found that this mTOR/S6K signaling plays an important role in regulation of food intake in the hypothalamus (80). pmTOR at Ser 2448 is highly localized in the PVN and ARC. pS6K1 at Thr389 is also colocalized with pmTOR mostly in neurons. They are found within about 90% of NPY/AgRP neurons and about 45% of POMC/CART neurons in the ARC. mTOR activity depends on the level of fuel, which is low in the ARC when the rats are fasted. Intraventricular administration of L-leucine, but not valine, decreases mTOR activity as well as food intake in 24-hour fasted rats, accompanied by a decrease in NPY. The study using the mTOR inhibitor, rapamycin, showed that the hypothalamic mTOR signaling is required for the decrease in food intake induced by L-leucine. The crucial role of hypothalamic mTOR signaling

as an intracellular fuel sensor extends to the anorectic action of other cytokines or agents. The hypothalamic mTOR signaling is also required for leptin-induced anorexia (80) and contributes to leptin resistance induced by diet-induced obesity (116). In addition, constitutive activation or inactivation of S6K in the mediobasal hypothalamus bidirectionally alters leptin sensitivity and food intake through the change of NPY and AgRP expression. Overactivation of S6K1 protects against high-fat diet-induced overeating (117). In contrast to this, there is a discrepancy of the role of mTOR activity between acute studies and chronic genetic model studies. It is reported that chronic activation of TSC1-mTOR pathway by targeted disruption of TSC1 in POMC neurons impairs anorectic action of POMC neurons and causes hyperphagia and obesity in mice (118). In spite of the strong evidences of the role of mTOR activity in regulation of food intake in the acute studies, the opposite results from chronic activation of mTOR in the POMC neurons might reflect the situations observed in obese mice. Another potent anorectic cytokine, ciliary neurotrophic factor also depends on the action of S6K1 (116). The anorectic action of fatty acid synthase inhibitors such as C75 and cerulenin is closely related to the hypothalamic mTOR signaling through utilization of neuronal glucose (69).

4.2. AMP-activated protein kinase (AMPK)

AMPK, a heterotrimer complex comprising a catalytic alpha submit and two regulatory beta and gamma subunits, is a master switch of energy balance in peripheral tissues as well as in the hypothalamus. AMPK within the ARC, PVN and VMN regulates food intake, but not in the LH, where leptin increases activated form of STAT3 (119, 120). Recently, a new conceptualization for regulation of AMPK was proposed (121). The ratio of AMP to ATP, representing the status of cellular energy, activates AMPK by allosteric regulation of AMPK. This leads to a change of balance between constitutive active LKB1 and dephosphorylation activity of protein phosphatse-2C alpha induced by AMP (122). Activation of Ca^{2+}/calmoduline-dependent protein kinase kinase alpha induced by Ca^{2+} phosphorylates AMPK independent of AMP (123). Increases in a complex of cell-death-inducing like-effector A (CIDEA) and beta subunit of AMPK produces ubiquitination-mediated proteolysis of AMPK independent of AMP or the process of phosphorylation and dephosphorylation (124). Although regulation of AMPK is not yet well studied at the upstream level, AMPK responds to leptin level as well as to nutritional status, which is closely associated with the hypothalamic neuropeptide system. Modulation of AMPK activity using constitutive active or dominant-negative expression of AMPK in the mediobasal hypothalamus regulates food intake and body weight (120). The activity of AMPK in the hypothalamus is also modulated by various factors. The anorectic factors such as feeding (120), leptin (120), insulin (120), glucose (120, 125), MC3/4 receptor agonist (120), fatty acid synthase inhibitor (70), alpha-lipoic acid (125), GLP-1 (126), CNTF (127), citrate (128) and high protein diet (129) all decrease the hypothalamic AMPK activity whereas orexigenic factors such as fasting (120), AgRP (120), ghrelin (119, 130), endocannabinoids (131), adiponectin (132) and glucocorticoids (133) all increase it. Interestingly, central or peripheral administration of resistin reduces food intake while it increases phosphorylation of hypothalamic AMPK (134).

AMPK plays a key role in integrating signals regarding regulation of food intake in the hypothalamus with mTOR. Both AMPK and mTOR often refer to "molecular fuel sensors" (120, 135). AMPK interacts with mTOR in regulation of food intake in the hypothalamus. Central administration of the AMPK agonist, 5-aminoimidazole-4-carboxamide-1-beta-D-ribose (AICAR), decreases phosphorylation of S6K and 4EBP1 at a dose that does not increase food intake while it increases phosphorylation of AMPK and ACC in the hypothalamus (136). ATP depletion directly or indirectly inhibits mTOR signaling via AMPK regulation of TSC2 (114). In addition, high protein diets and central administration of leucine reduces food intake via decreased phosphorylation of AMPK and increased mTOR signaling pathways (129). Pretreatment with leucine in the hypothalamus at the dose that does not reduce food intake inhibits 2-DG-induced increase in food intake and phosphorylation of AMPK, indicating that mTOR activity regulates food intake via AMPK in the hypothalamus (129). Therefore, AMPK and mTOR have a bidirectional reciprocal inhibitory action in regulation of food intake (Figure 1).

AMPK regulates energy balance in the hypothalamus via AMPK-malonyl-CoA-CPT1 axis (119) similar to what occurs in peripheral tissues (122). Acetyl-CoA carboxylase (ACC), which regulates fatty acid metabolism, is a main target for AMPK. The activation of AMPK induced by fasting and ghrelin subsequently phosphorylates acetyl-CoA carboxylase (ACC), which leads to decrease the conversion of acetyl-CoA to malonyl-CoA and decreases gene expression of fatty acid synthase via SREBP1-dependent mechanism in the hypothalamus. This leads to decreased concentration of malonyl-CoA in the hypothalamus and increased activity of carnitine:palmitoyl-CoA transferase-1 (CPT-1) on the outer membrane of mitochondria, a rate-limiting step for increasing fatty acid oxidation by enhancing the entry of long-chain acyl-CoA into mitochondria (119). The net outcome of activated AMPK in the hypothalamus increases food intake. The inactivation of AMPK induced by C75, a fatty acid synthase inhibitor, also decreases food intake, possibly by decreasing NPY expression in the hypothalamus via inactivation of cAMP response element-binding protein (CREB) (70) (Figure 1).

The direct interaction of AMPK with Akt/PKB in the hypothalamus remains unclear. A high protein diet and leucine do not change the Akt/PKB activity induced by insulin in the periphery (129). However, Akt1 prevents activation of AMPK via LKB1 or other AMPK kinase in the heart (137). Adiponectin induces the phosphorylation of AMPK, Akt/PKB, and endothelial nitric oxide synthesis in human umbilical vein endothelium cells (HUVECs). Dominant-negative AMPK inhibits the phosphorylation of Akt/PKB induced by adiponectin indicating that AMPK is upstream of Akt/PKB (138). Therefore, the cross-talk

between AMPK and Akt/PKB in the hypothalamus is possible in the regulation of food intake.

5. CONCLUSION

Energy balance is tightly regulated by coordinating and integrating signals from the periphery in key circuits within the CNS. Some pharmacologic agents, nutrients, and hormones have been introduced to affect food intake by a direct action within the hypothalamus. However, the underlying mechanism of anorectic or orexigenic action is different among the various factors. The classical hypothalamic neuropeptide system has been demonstrated to be a key mechanism by which certain substances affect food intake in the hypothalamus. Recent studies about AMPK from Kahn's laboratory as well as mTOR from our laboratory as being key CNS fuel sensors open a new way to demonstrate how the energy balance is regulated via intracellular cascades in neurons within the hypothalamus. These intracellular molecules participate in regulation of food intake by interacting with other kinases involved in different signaling pathways. Akt/PKB is a key intracellular molecule for the insulin signaling pathway and communicated with other kinases such as mTOR and IKK beta. Akt/PKB is also shared with other important cytokines such as leptin. However, the role of Akt/PKB and its relationship with newly-emerging signaling molecules involved in regulation of energy balance remains unclear. Precise molecular mechanisms at the cellular level will allow us to understand how energy balance is specifically regulated within the CNS.

Given the rapid increase in the prevalence of obesity and subsequent metabolic disease, there are only a few way to meet the serious situation. The understanding of the CNS network regarding energy balance at the molecular level will offer us scientific evidences to develop a pharmacologic agent or other ways such as genetic modulation without undesired effects. Therefore, scientific efforts to reveal the CNS sensing mechanism will provide new opportunities to fight against obesity and metabolic disease.

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Abbrevations: ACC: Acetyl-CoA carboxylase; AICAR: 5-aminoimidazole-4-carboxamide-1-β-D-ribose; Akt/PKB: Akt/protein kinase B; AMPK: AMPK-activated protein kinase; AP: area postrema; ARC: arcuate nucleus; CCK: cholecystokinin; CIDEA: cell-death-inducing like-effector A; CPT-1: carnitine:palmitoyl-CoA transferase-1; CREB: cAMP response element-binding protein; FAS: fatty acid synthase; FoxO1: forkhead box O1; IR: insulin receptor; IREs: insulin responsive elements; IRS: insulin receptor substrate; JAK2: Janus kinase; Lepr: leptin receptor; LeprB: long form of Lepr; LHA: lateral hypothalamic area; MC4: melanocortin-4; MCD: malonyl-CoA decarboxylase; MCH: melanin-concentrating hormone; mTOR: mammalian target of rapamycin; NAc: nucleus accumbens; NIRKO: neuron-specific insulin receptor knockout; NPY/AgRP: neuropeptide Y/agouti-related peptide; NTS: nucleus tractus solitaries; PDK1: phosphoinositide dependent protein kinase-1; PFA: perifornical area; PFC: prefrontal cortex; PI3K: phosphatidylinositol-3-OH kinase; PIP2: phosphatidylinositol-4,5-bisphosphate; PIP3: phosphatidylinositol-3,4,5-trisphosphate; POMC: proopiomelanocortin; Pten: phosphatidylinositol-(3,4,5)-triphosphate phosphatase; PTP1B: protein tyrosine phosphatase 1B; PVN: paraventricular nucleus; Rheb: ras homolog enriched in brain; SF1: steroidogenic factor 1; SOCS3: suppressor of cytokine signaling; STAT3: signal transducer and activator of transcription; TSC2: tuberous Sclerosis Complex protein 2; VMH: ventromedial hypothalamus.

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Send correspondence to: Dong-Hoon Kim, Department of Pharmacology, Korea University College of Medicine, 126-1, 5-Ga, Anam-Dong, Seongbuk-Gu, Seoul 136-705, Republic of Korea, Tel: 82-2-920-6237, Fax: 82-2-920-0824, E-mail: LDHKIM@korea.ac.kr