Central Nervous System Nutrient Signaling: The Regulation of Energy Balance and the Future of Dietary Therapies

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Annu. Rev. Nutr. 2010. 30:219–35

First published online as a Review in Advance on March 12, 2010

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

This article's doi: 10.1146/annurev.nutr.012809.104723

0199-9885/10/0821-0219$20.00

Key Words

hypothalamus, arcuate, mTOR, AMPK, leucine, S6K1

Abstract

The mammalian target of rapamycin (mTOR) pathway coordinates cell growth in response to nutrient availability. Increasing evidence points to a role for mTOR to also direct whole-body energy balance in response to micronutrient as well as hormonal cues. This positions mTOR as a key central integrator of acute and chronic changes in fuel status. Energy balance is affected by mTOR in several organ systems, including the hypothalamus, where the pathway can modulate feeding. We propose that a greater understanding of this nutrient-sensitive pathway may open the door to more intelligent, effective diet design based on the effects of micronutrients on specific signaling pathways.

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DESIGNING A DIET FROM THE GROUND UP: LESSONS FROM CELLULAR NUTRIENT SENSING

Millions of Americans are engaged in an ongoing battle to lose weight. Much of that battle is waged at the dinner table, where individuals are constantly trying to choose foods that will help them lose weight and improve other aspects of their health. The reality for most people is that these dietary choices are made by following the latest fad diet. In the majority of cases, these diets lack any critical scientific data that support their use or even justify the choices of both macro- and micronutrients. Obviously, most of these diets fail. In general, these diets fail because they do not overcome the homeostatic systems that serve to regulate body weight.

What we propose is a different potential approach to diet design. The past decade has seen an explosion in our understanding of the signaling abilities of individual micronutrients. More than simply acting as a source of energy, individual micronutrients can activate distinct signaling pathways. These different signaling pathways regulate different cellular processes. Consequently, this new information makes it possible to link specific micronutrients to particular biological effects. We suggest that knowledge about the signaling properties of these nutrients may allow for diets to be designed with specific outcomes in mind. This includes reducing the burden of metabolic diseases.

This review focuses on fuel sensing in the hypothalamus, a brain region known to direct ingestive behavior and peripheral metabolism. We describe mechanisms for cellular nutrient sensing and translation of these signals into meaningful patterns of neuronal activity. Specifically, we focus on the mammalian target of rapamycin (mTOR) as a cellular fuel sensor that has been adapted as a key component of the energy balance regulatory system.

REGULATION OF ENERGY BALANCE: NEUROENDOCRINE SYSTEMS

Adipose mass is regulated by a complicated neuroendocrine system that works to adjust caloric intake and expenditure to maintain body fat. A key component of that system is the hormone leptin, which is made in adipose tissue and circulates in proportion to the amount of body fat (14). Leptin provides crucial information to the central nervous system (CNS) about the status of adipose tissue via receptors in a number of regions, including the arcuate nucleus (ARC) of the hypothalamus. The ARC is composed of

two neuronal populations thought to be important effectors of hormonal and local fuel signaling. The first population contains catabolic pro-opiomelanocortin (POMC)-producing neurons. POMC mRNA expression is increased in the ARC by leptin and insulin (11, 64, 87). POMC is cleaved to produce alpha melanocyte-stimulating hormone (α-MSH), a peptide whose role in peripheral cells is to regulate skin and hair pigmentation. Exogenous α-MSH administration has been shown to decrease food intake, producing weight loss (23, 96, 97); this effect is mediated by the MC4 melanocortin receptor subtype, found concentrated in the hypothalamus. Increased food intake and body weight are observed in MC4-deficient mice (37), supporting a role for endogenous α-MSH to affect energy homeostasis.

A second population produces the anabolic peptide transmitters Agouti-related peptide (AgRP) and neuropeptide Y (NPY). AgRP is found exclusively in the ARC and acts as a competitive antagonist/inverse agonist at MC4 receptors (33). During times of energy deficiency, AgRP blocks the catabolic effects of α-MSH, resulting in increased food intake and in weight gain. Exogenous AgRP administration or genetic AgRP overexpression has been shown to produce weight gain and to stimulate food intake (29, 84). Interestingly, however, genetic disruption of AgRP has no effect on either food intake or weight gain (81). Like AgRP, NPY also stimulates food intake and weight gain (10, 30).

CENTRAL NERVOUS SYSTEM NUTRIENT SENSING

Dietary treatment of obesity requires an understanding of the impact of particular nutrients on energy homeostasis. Fuel monitoring, the ability to sense glucose and other nutrients, maximizes a cell's ability to carry out cellular functions. In the brain, fuel monitoring adjusts food intake behavior in response to nutrient availability for the organism. The ARC houses neuronal circuitry for this purpose. Like other individual peripheral cells, these neurons monitor local nutrient supply in order to maintain their own energy supplies in the face of fluctuating metabolic substrate availability. These specialized neurons may also exploit these same fuel-sensing mechanisms in order to affect whole-body energy balance (**Figure 1**). This is accomplished by modulating neural pathways that control food intake and/or energy expenditure.

Neurons rely almost exclusively on glucose for energy that is derived from peripheral digestion and catabolism. It is not surprising, therefore, that neurons have been proposed to monitor peripheral glucose availability. Over 50 years ago, Jean Mayer (59) outlined the "glucostatic hypothesis," proposing that "glucoreceptors" in the hypothalamus sense arterio-venous fluctuations in blood glucose in order to modify food intake. Later, "glucose responsive," or glucose-excited (GE), and "glucose sensitive," or glucose-inhibited (GI), neurons were identified in the hypothalamus (75). In these select neurons, glucose is not merely a metabolic substrate, but also a signaling molecule that controls neuronal firing rate.

Key support for Mayer's hypothesis is based on reports of glucoprivic feeding. Administration of the glucose analogue 2-DG arrests glycolysis and elicits a strong feeding response (7). Glucose-sensing neurons in hypothalamic feeding centers have been hypothesized to be responsible for regulating intake in response to ambient glucose levels. In the ARC, glucose sensing is thought to occur in both AgRP/NPY and POMC neurons. Signaling mechanisms elicited by glucose in each type of cell may not be identical, meaning that a unidirectional change in glucose availability must simultaneously produce bidirectional responses in different cell types. Both POMC and AgRP/NPY neurons are known to respond to changes in ambient glucose levels (38), but POMC neurons are classified as glucose-excited cells, whereas AgRP/NPY neurons are classified as glucose-inhibited cells (70).

Glucosensing neurons may implement mechanisms used by pancreatic β-cells.

Specialized fuel-sensing neurons contain the rate-limiting glycolytic enzyme glucokinase (GK) (56). GK in glucose-responsive neurons translates ambient glucose into an ATP:ADP ratio that may act on downstream fuel sensors to affect neuronal activity. Because the K_m of GK is within the physiologic range for blood glucose, unlike neuronal hexokinase, GK is not subject to feedback inhibition over the normal blood glucose range. Therefore, very small, intermeal fluctuations in blood glucose may produce substantial changes in cytoplasmic ATP:ADP ratios, which might be translated into a signal that is meaningful to these specialized neurons. Rats deficient for GK exhibit hyperphagia (109), but it is unclear whether this effect is due to GK action in hypothalamic neurons. Levin and colleagues (20) report that feeding is unaffected after GK activity is altered in the ventromedial hypothalamus, which includes the ARC.

ATP-sensitive K^+ (K_{ATP}) channels may titrate neuronal activity in response to ambient glucose. In β-cells, GK regulates the rate of ATP production from glucose, thereby controlling K_{ATP} channel closure (58). In the CNS, ATP generated during glucose metabolism may act on K_{ATP} channels to alter neuronal firing rates. K_{ATP} channels in the brain are located on neurons but are absent from glia (19). Like pancreatic β-cell K_{ATP} channels, neuronal K_{ATP} channels are inward-rectifying K^+ channels whose inhibition or closure in response to ATP elicits Ca^{2+} influx and cellular depolarization and whose activation, a response to phosphorylation, elicits cellular hyperpolarization (53). Glucose-induced changes in the firing rates of GE neurons appear to be mediated by the closure of K_{ATP} channels under high glucose conditions. Closure of the channel results in increased intracellular K^+ and subsequent membrane depolarization. In this manner, GE neurons are much like pancreatic β-cells, which depolarize and secrete insulin in response to elevated blood glucose levels.

Due to their ability to affect the excitability of POMC and AgRP/NPY neurons, K_{ATP} channels are known to play a role in the regulation of both blood glucose levels and ingestive behavior. When the ATP-mediated closure of K_{ATP} channels in POMC neurons is disrupted via selective expression of a mutant Kir6.2 subunit, mutant mice display impaired glucose tolerance and an attenuated glucose-stimulated α-MSH release from POMC neurons (76). Glucose intolerance in this model may be due to overabundant hepatic gluconeogenesis. Pharmacologic activation of the K_{ATP} channel hyperpolarizes the neuron and has been found to inhibit hepatic gluconeogenesis (79). On the other hand, pharmacologic inhibition or genetic impairment of the channel depolarizes neurons and blocks hepatic glucose production in response to centrally administered insulin (79).

Less is known about the role of K_{ATP} channels in the regulation of food intake behavior. Ablation of the K_{ATP} channel subunit Kir6.2 has been demonstrated to reduce glucoprivic feeding (61). Furthermore, animals deficient for the phosphatidylinositol-(3,4,5)-trisphosphate phosphatase Pten in POMC neurons are unresponsive to the anorectic effects of leptin and demonstrate increased K_{ATP} channel activity. Tolbutamide, a K_{ATP} channel blocker of the sulfonylurea family, restores leptin-evoked firing of neurons when applied in vitro and abolished hyperphagia in POMC-specific Pten knockout mice when administered intracerebroventricularly (icv) (78). Other studies have found that leptin inhibits hypothalamic neurons via the activation of K_{ATP} channels (95) and that leptin activates ARC neurons in a phosphoinositide 3-kinase (PI3K)-dependent manner (63). Collectively, these data support a link between K_{ATP} channel activity and the regulation of ingestive behavior. These data also suggest a mechanism for the glucose-dependent actions of leptin to reduce food intake.

Modulation of neuronal activity by glucose may be due either to a direct action on neurons or to an indirect effect of its metabolism in astrocytes. Astrocytes metabolize glucose anaerobically, releasing lactate that is taken up by neurons via monocarboxylate transporter-1 and converted into pyruvate

for ATP-producing mitochondrial oxidation. Monocarboxylate transporter-1 is known to be expressed in hypothalamic neurons, where lactate can alter the activity of glucose-sensing neurons (65, 94, 108). Lactate's actions are presumably due to the effects of its metabolite, pyruvate. Glucoprivic conditions typically elicit food intake, but pyruvate may mimic the effects of glucose replacement under these conditions. Centrally administered pyruvate can suppress a 2-DG-induced stimulation of food intake (50). This effect is presumably due to the ability for pyruvate to alter the activity of melanocortin neurons. When added to a neuronal cell line expressing AgRP or to 2-DG-treated hypothalamic explants, pyruvate decreases AgRP expression (50). Pyruvate thus appears to act downstream of glucose to indicate fuel availability and inhibit the activation of orexigenic neurons. A potential mechanism for the glucomimetic action of pyruvate is the generation of ATP, which can act on downstream fuel sensors [e.g., adenosine monophosphate-activated protein kinase (AMPK) and/or mTOR] known to affect hypothalamic function.

Neurons in these same glucose-responsive regions appear also to sense the availability of nonglucose nutrients. Although neurons are not known to use fatty acids for energy, it is hypothesized that neuronal fatty acid synthesis and/or oxidation in certain brain regions provides a read-out of local fatty acid availability in order to effect changes in food intake behavior. In fact, both POMC and AgRP neurons are known to respond to local fluctuations in fatty acid levels. Icv oleic acid inhibits NPY/AgRP expression in the hypothalamus (73). Furthermore, central administration of the fatty acid synthase inhibitor C75 reduces food intake by increasing POMC expression (92) and decreasing AgRP expression (55) in the ARC.

MAMMALIAN TARGET OF RAPAMYCIN AS A WHOLE-BODY FUEL SENSOR

mTOR is an atypical kinase known to coordinate cell growth by regulating protein synthesis in response to nutrient availability. Over the past few years, data have also described mTOR as a crucial player in the control of whole-body energy homeostasis. This regulation may manifest as changes in food intake behavior and/or as altered sensitivity to insulin and/or leptin. Recent research has positioned the hypothalamic mTOR pathway at the crossroads of nutrient and hormonal signaling in order to integrate minute-to-minute and long-term energy status. In this way, manipulation of hypothalamic mTOR signaling may provide a basis for novel obesity therapies effective in the face of leptin and insulin resistance.

It has been known for decades that cells will grow to a certain size prior to division. Because uncontrolled proliferation would produce infinitely smaller cells, this requirement allows organisms or organs to grow while preserving cell size. Regulation of the growth response is controlled in part by mTOR, a downstream effector of PI3K. In the *Drosophila* wing, overexpression of a dominant-negative PI3K allele reduced wing cell size and number, whereas overexpression of the wild-type allele had the inverse effect (52). This cell size phenomenon can be recapitulated by mutation of the downstream target of mTOR S6K1 (66). This pivotal study identified mTOR as a critical regulator of cell growth in normal mammalian cells.

The growth-promoting properties of mTOR activation are due to its ability to regulate transcription, translation initiation and elongation, and cell-cycle progression (54, 107). mTOR is a component of two multiprotein complexes. One is mTOR complex 1 (mTORC1), which contains the protein raptor, G protein β-subunit-like protein (GβL), and protein kinase B. The other is mTOR complex 2 (mTORC2), containing the protein Rictor, GβL, and mammalian stress-activated protein kinase. Although both complexes are stimulated by mitogens via the PI3K pathway, only mTORC1 appears to be nutrient dependent (99). In response to low intracellular energy availability, mTORC1 stabilizes and reduces the activity of mTOR kinase. Nutrient

abundance, on the other hand, promotes activity of the mTOR kinase, leading to the direct phosphorylation of S6K1. S6K1 promotes protein synthesis via the phosphorylation of transcriptional machinery such as S6 ribosomal protein (S6R), EIF4E binding protein, and eukaryotic elongation factor 2 kinase.

Analogous to the way that individual cell growth is coordinated by fuel availability, whole-organism metabolism is also constantly regulated by nutrient flux. mTOR is a critical fuel sensor contributing to this coordination. About 12 years ago, Shima and colleagues (91) noticed that S6K1-/- mice were smaller and leaner than were their wild-type littermates. More recently, it was found that when exposed to a high-fat diet, these mice are protected against diet-induced obesity (100). These data promote a role for mTOR not only to govern cell growth, but also to regulate whole-body expansion. Energy balance regulation by mTOR is presumably due to the coordination of metabolic processes in multiple organs. Given this role for mTOR as a coordinator of nutrient- and hormone-mediated metabolic processes, it was hypothesized that mTOR might affect energy homeostasis by acting in key regulatory areas of the CNS. Data to support this hypothesis come from our group (15, 16, 80) and others (6, 68). Cota and colleagues (16) found physiologic regulation of the pathway in hypothalamic feeding centers. Their initial prediction was based on data linking mTOR activity to the development of cancer, diabetes, and obesity (39, 57). Like most mammalian cells, ARC neurons also express mTOR. However, mTOR and its downstream kinase, S6K1, are phosphorylated only in POMC and AgRP neurons within the ARC. After three hours of refeeding in rats fasted for 45 hours, Cota et al. (16) observed increased phosphorylation of pmTOR at Ser2448, pS6K1 at Thr389, and pS5 at Ser240 and Ser244 in ARC neurons. Although 90% of ARC NPY/AgRP neurons express pmTOR and pS6K1, only 45% of ARC POMC/CART neurons reveal phosphorylated pmTOR and pS6K1. S6K1 is widely expressed in the CNS (2), but it is unknown whether activation of the kinase may be regulated nutritionally in extra-ARC energy balance centers.

Importantly, mTOR activity in these areas is linked to the regulation of ingestive behavior. The potent mTOR activator leucine crosses the blood-brain barrier (93) and therefore can access the brain when ingested after a meal. Cota et al. (16) hypothesized that icv leucine would produce anorexia if central mTOR signaling mediates the anorectic response to high-protein feeding. Indeed, central leucine delivery produced rapamycin-sensitive reductions in food intake. This effect was not observed following icv L-valine, which does not drive mTOR pathway activity. This nutrient-driven pathway appears also to drive leptin-induced anorexia, introducing the possibility that central mTOR may integrate local and peripheral energy cues.

Cota's findings are supported by the earlier finding that S6K1 overexpression in *Drosophila* insulin-like peptide (DILP)-producing neurons suppresses ingestive behavior in fasted larvae (106). Although these data point to a role for mTOR to alter central melanocortin signaling, they do not describe a downstream mechanism by which mTOR might alter behavior. More recently, Blouet et al. (6) demonstrated that bilateral expression of a constitutively active form of S6K1 in the mediobasal hypothalamus (MBH) not only decreased food intake and body weight but also reduced the expression of NPY and AgRP mRNA in the region. MBH POMC mRNA levels were unaltered by the manipulation, suggesting that S6K1 activation might act specifically to inhibit AgRP/NPY neurons in the MBH in order to suppress food intake. Interestingly, expression of a dominant-negative form of the kinase in the same region altered expression of none of the three transcripts. The latter finding might suggest that S6K1 drives changes in hypothalamic neuropeptide expression only during the presence of a stimulus, for example, following a meal. The authors also report that expression of constitutively active S6K1 in the MBH suppresses energy expenditure by decreasing oxygen consumption and

respiratory exchange ratio, an effect that is likely to be a direct consequence of the anorexia also observed in these animals.

Data from Mori et al. (68) also link hypothalamic mTOR activity to downstream melanocortin signaling. Deletion of the negative regulator of mTOR, Tsc1, in POMC neurons resulted in dysregulated expression of key neuropeptides, including POMC and NPY. Conditional knockouts failed to exhibit either increased POMC expression or attenuated NPY expression during feeding. Regulation of AgRP expression remained normal. It should be highlighted that the latter finding seems at odds with these other observations about how this fuel-sensitive pathway influences the key circuits to regulate energy balance. However, it should be noted that the activity of the mTOR pathway maybe critical to the development of these POMC neurons and that the resulting phenotype may be due to a developmental defect.

The ability for mTOR to monitor an organism's fuel status depends on an ability to sense both acute and chronic changes in energy availability. By acting on a common substrate (mTOR), this information may be integrated to alter homeostatic variables. As discussed above, nutrients can have direct effects on feeding behavior. Additionally, nutrients can act indirectly to alter hormone action, with mTOR being one mechanism by which nutrients can exert this effect.

mTOR INTEGRATES MIXED MICRONUTRIENT SIGNALS TO INFLUENCE ENERGY BALANCE

Responding to nutrient signals is one way in which energetic substrates like mTOR may regulate energy balance in response to systemic fuel availability. In a number of models, mTOR activation requires glucose availability. In a human breast cancer cell line, phosphorylation of mTOR and its downstream targets, S6K1 and 4EBP1, is decreased after addition of the glucose analogue, 2-DG (40). In isolated working rat hearts, activation of the mTOR pathway is increased after the addition of glucose, and this activation is rapamycin sensitive, as measured by phosphorylation of mTOR and S6K1 (89). Glucose is also required for the activation of the mTOR pathway following perfusion of the heart with insulin.

It is unclear what mechanism may underlie mTOR's glucose requirement. One hypothesis (102) proposes that the hexosamine biosynthetic pathway (HBP) may be responsible for glucose-induced mTOR activation. Glucose-induced stimulation of mTOR is blocked by an inhibitor of the HBP rate-limiting enzyme glucose-fructose amino transferase. Glucosamine augments HBP flux and mimics glucose, activating mTOR. Another hypothesis points to the K_{ATP} channel as a critical intermediate. In cultured β-cells, inhibition of K_{ATP} channels with glyburide has been shown to result in the K_{ATP}- and Ca^{2+}-dependent phosphorylation of mTOR's downstream kinase, S6K1, at basal glucose levels (49). Others (51) suggest a permissive role for glucose to activate mTOR. According to this report, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) binds to Rheb under low-glucose conditions, preventing mTOR activation. When glycolytic flux is restored, Rheb is relieved from negative regulation by GAPDH, and mTOR may be activated. This phenomenon persists in TSC1-deficient and AMPK-silenced cells, arguing that GAPDH interacts directly with Rheb in mTORC1.

After a mixed meal, circulating levels of both carbohydrates and lipids rise. As a result, it is not surprising that neuronal responses to these signals might be intimately related. Nearly 50 years ago, Randle et al. (82) described a reciprocal relationship between glucose and fatty acid metabolism, whereby promoting the former will inhibit the latter and vice-versa. Recent evidence supports a role for Randle's effect to mediate mTOR's sensitivity both to lipids and to glucose. In the heart, oleic acid has been demonstrated to increase the phosphorylation of mTOR's downstream effector, S6K1 (89), and this effect is thought to be due to glycolytic inhibition. Fatty acid

synthase inhibition activates the mTOR pathway (80), an effect that requires intracellular glucose. Ketogenic diet feeding depletes neuronal glucose availability and abrogates the anorectic and mTOR-activating effects of centrally administered C75 (80, 105). Additional evidence suggests that mTOR signaling may in fact contribute to the Randle effect: mTOR activity may feed back to promote fatty acid synthesis. In human breast cancer cells, human epithelial growth factor receptor-2 promotes fatty acid synthase and ACCα expression in an mTOR- and PI3K-dependent manner (110).

Coordination of protein synthesis in response to cellular amino acid content is another role for cellular fuel sensing. mTOR is an important nutrient sensor for this purpose. A role for amino acids to control mTOR pathway activation was first described by investigators interested in macroautophagy (46). Addition of amino acids to cells inhibits macroautophagy, an effect that is rapamycin sensitive (5). Branched chain amino acids (BCAAs) (34, 45), especially leucine (90), activate mTOR more potently than do other amino acids. During nutrient deprivation, BCAAs can rescue suppression of the mTOR pathway by destabilizing an interaction between mTOR and its inhibitory molecule, raptor (42).

For some time, it has been known that protein intake can prevent weight gain. High-protein diets may promote thermogenesis and satiety (28, 31). In rats, supplementation of high-fat diets (HFDs) with BCAAs reduces food intake and weight gain (72), whereas a low-protein diet increases food intake and decrease hypothalamic AgRP expression (69).

Recently, many of the metabolic effects of high-protein diets have been attributed to mTOR signaling in the brain and peripheral tissues. A recent report from Ropelle et al. (83) demonstrates that decreased adiposity and reduced food intake in rats fed a high-protein diet are associated with decreased AMPK and increased mTOR signaling in the hypothalamus. These effects are mTOR-dependent, as they were diminished after the infusion of an antisense oligonucleotide directed toward mTOR. The high-protein diet also decreased hypothalamic NPY expression while increasing hypothalamic POMC messages in both fed and fasting conditions; these effects persist in *ob/ob* mice. The link between hypothalamic mTOR activity and melanocortin signaling is supported by work in cultured hypothalamic neurons, where rapamycin can increase AgRP expression (69).

Dietary amino acids appear to play a protective role against obesity by activating mTOR, but the role of this pathway to counteract obesity in overweight individuals remains unclear. Several groups have demonstrated increased plasma amino acids in obese subjects (24–26, 72). If BCAA supplementation promotes anorexia and weight loss, then why is it that these obese subjects remain obese? One possibility is that the protective anorectic effect of central mTOR activation may be overshadowed by insulin resistance that results from mTOR activation in peripheral tissues. In fact, a twofold rise in serum amino acid concentration corresponds to a 25% reduction in insulin sensitivity (48), perhaps contributing to obesity.

Newgard and colleagues (72) demonstrate that BCAA supplementation exacerbates insulin resistance in HFD-fed rats, and that rapamycin restores sensitivity in these animals. The effect was not observed in rats fed BCAA-supplemented chow, suggesting an interaction between amino acids and lipids to promote insulin resistance. During HFD feeding, BCAAs might overwhelm an already saturated mitochondrial oxidative capacity. In the same manner, amino acids may compete with glycolytic products for mitochondrial β-oxidation (47), contributing to hyperglycemia. Together, these data suggest that overnutrition can overwhelm metabolic pathways that are designed to maintain energy homeostasis. In the absence of overnutrition, BCAAs may promote homeostasis via activation of the mTOR pathway. When HFD is supplemented by leucine alone, improved glucose homeostasis has been observed (111). This latter effect might be due to a lower level of overnutrition in these rats.

The ability for BCAAs and mTOR activity to simultaneously modulate feeding behavior and insulin sensitivity highlights the importance of understanding the differential effects of central versus peripheral mTOR activity. Chronic, intraperitoneal (ip) rapamycin treatment provides protection against diet-induced obesity and diabetes in mice (9). Activation of the mTOR pathway in muscle and β-cells promotes insulin resistance; peripherally administered rapamycin counteracts the effects of HFD feeding to promote insulin resistance by attenuating mTOR signaling. In this study, ip rapamycin treatment was associated not only with decreased body weight and fat mass, but also with reduced serum insulin and plasma glucose levels and reduced rate of fatty liver disease. Additional evidence suggests that rapamycin might reduce adipogenesis (4) by reducing expression of adipogenic transcripts (12). However, consistent with a centrally mediated effect, rapamycin-treated mice are also hyperphagic (8). These divergent actions must be considered when designing any potential therapy, pharmacologic or dietary, targeting the mTOR pathway.

AMPK AND mTOR: TANDEM SYSTEMS

mTOR and AMPK are inversely and simultaneously regulated, forming a bidirectional control system linking neuronal fuel availability and function. Activity of each of the two kinases fluctuates inversely in response to a given energetic manipulation. Coordinated regulation of the two substrates appears to be due to two important interactions between them. The first is a direct phosphorylation of mTOR on Thr2446 by AMPK; this phosphorylation event appears to prevent the phosphorylation of Ser2448. The second interaction is the phosphorylation of tuberin by AMPK. Tuberin, also known as TSC2, is a GTPase-activating protein that suppresses mTOR activity by increasing relative GDP binding to the mTOR complex component, Rheb (44).

AMPK is a nutritionally regulated target that appears to link homeostatic responses to both nutrients and hormone. AMPK activity decreases when energy, including glucose, is abundant, and this regulation has been demonstrated in ARC, among other brain regions (50, 62). AMPK activity is altered during fasting and feeding and is also thought to be responsible for anorectic responses to a number of circulating hormones, including leptin (27, 35, 62). AMPK's catalytic subunits colocalize with NPY in ARC (43), and NPY expression is stimulated by genetic (62) or pharmacologic (1) activation of AMPK.

Like mTOR, AMPK is highly responsive to changes in cellular glucose content. AMPK is required for the acute electrophysiological responses of GE POMC neurons and GI AgRP neurons to changes in glucose concentration. Selective deletion of AMPK in AgRP and POMC neurons impairs the normal, acute electrophysiological excitation of AgRP neurons under low-glucose conditions and of POMC when glucose is elevated (13).

AMPK activity is also sensitive to changes in fatty acid availability. In fact, AMPK is a key regulatory element of the fatty acid synthesis machinery. Acetyl-CoA carboxylase (ACC) is phosphorylated and inhibited by activated AMPK (104). ACC uses acetyl-CoA from glucose metabolism to synthesize malonyl-CoA. Due to a requirement for its substrate, Acetyl-CoA, and to inhibition of ACC under low ATP conditions, malonyl-CoA is produced only when neuronal energy availability is abundant. Because local malonyl-CoA depletion can decrease AgRP and NPY expression in the hypothalamus (36), it may provide a downstream mechanism for the modulation of ARC neuronal function in response to altered AMPK activity.

The ability for AMPK to directly phosphorylate mTOR may alter the nutrient sensitivity of the latter substrate. This hypothesis is supported by the finding that palmitate-induced S6K1 phosphorylation in cultured hepatocytes is metformin sensitive and therefore likely AMPK dependent (67). It is unclear whether

an AMPK–mTOR interaction underlies all of the metabolic effects of each of these substrates, but it is clear that the two substrates interact simultaneously and reciprocally to affect energy balance.

ROLE FOR CENTRAL mTOR SIGNALING TO MEDIATE HORMONE-INDUCED ANOREXIA: mTOR AS A MECHANISTIC EXPLANATION FOR THE NUTRIENT DEPENDENCE OF CENTRAL HORMONAL SIGNALS

As a central fuel sensor, hypothalamic mTOR activity modulates feeding behavior in order to direct whole-body expansion in response to nutrient availability. As discussed above, circulating nutrients such as amino acids, glucose, and lipid may serve as a read-out of whole-body fuel status. Hormonal cues, however, are also important signals of energy balance and may reflect meal-independent measures of fuel status, such as adiposity. Increasing evidence points to the ability for hypothalamic mTOR to respond to hormonal cues, mediating the anorectic response to hormones such as leptin. This could potentially extend to GLP-1, whose action in β-cells appears to require mTOR (49). Hypothalamic mTOR might therefore act to integrate hormone- and nutrient-based cues regarding energy balance in order to modulate melanocortin signaling in a manner that reflects both the short- and long-term fuel status of the organism.

LEPTIN

Leptin binding to the long form of its receptor (ObR) induces activation of Janus kinase 2 (JAK2), which binds and phosphorylates STAT3 (18, 86). STAT3 acts in the nucleus to regulate transcription of several genes implicated in the control of energy balance (86). ObRb is highly expressed in the ARC (22, 60, 85), where it inhibits NPY/AgRP neurons and activates POMC neurons (17, 21, 88), as well as in other, extra-ARC brain regions (22). Normally, leptin serves as an adipostatic signal to produce anorexia, but individuals rendered obese via overfeeding are usually leptin resistant.

In 2006, Cota and colleagues (16) demonstrated activation of the hypothalamic mTOR pathway following injection of central leptin. Co-injection of rapamycin abrogated not only mTOR activation but also the anorectic response to leptin, suggesting that mTOR activity must be required for leptin-induced anorexia. Later (15), the group demonstrated the loss of S6K1 activation after leptin injection in rats fed HFD for four weeks. Resistance to leptin is unique in this paradigm, as icv injection of ciliary neurotrophic factor potently activates mTOR substrates following an identical feeding regimen. Thus, HFD feeding must not impair the mTOR pathway itself, but only the ability for certain ligands (such as leptin) to activate the pathway. Basal S6K1 phosphorylation is reduced in the rat hypothalamus after both one day and four weeks of HFD feeding (16), but this effect is most likely due to reduced sensitivity to endogenous circulating factors in the obese rodents rather than to an intrinsic defect in the mTOR pathway.

A recent report suggests a more complicated story, however. As predicted, leptin deficiency and fasting both reduced ventromedial hypothalamus mTORC1 activity, as measured by phosphorylation of S6R (101). However, these same manipulations stimulated phosphorylation of S6R in the ARC. It is clear from these inconsistencies surrounding the regulation and function of mTOR signaling in the hypothalamus that much remains to be learned. The key take-away point from these studies is the understanding that mTOR signaling may vary according to cell type and, depending on the conditions, may not respond uniformly to given stimuli.

GUT-DERIVED HORMONES: GLP-1 AND GHRELIN

Incretins, including GLP-1, are released from the gut in response to glucose. The hallmark

role of GLP-1 is as an insulin secretagogue; in the presence of glucose, GLP-1 enhances insulin secretion from pancreatic β-cells. Interestingly, this mechanism might require the glucose-sensitive mTOR pathway. The GLP-1 receptor agonist, Exenatide, enhances glucose-stimulated S6K1 phosphorylation in cultured β-cells (49). A similar effect is observed after addition of an analogue of the GLP-1 downstream mediator, cAMP. These data provide indirect evidence that mTOR may play a role to mediate the effects of GLP-1 on insulin secretion. It is unknown whether mTOR may be required for the effects of central GLP-1 on feeding behavior and glucose homeostasis. Exploration of this potential relationship in vivo should be a research priority.

Another gut-derived hormone, ghrelin, also appears to interact with mTOR signaling. Ghrelin is secreted from oxyntic cells of the stomach and activates AgRP neurons in the hypothalamus to elicit hyperphagia (71). Recently, icv ghrelin was demonstrated to induce phosphorylation of S6K1 in MBH, where it also elicited neuronal activation, as measured by c-fos immunoreactivity (101). Because ghrelin characteristically activates AgRP neurons in this region, it can be interpreted from these data that ghrelin may stimulate mTOR activity in AgRP neurons. This must be tested directly, however. Additionally, it must be noted that although ghrelin induced S6K1 phosphorylation and c-fos in large populations of cells, the colocalized population was quite small, meaning that S6K1 activation may be incidental in some neurons.

INSULIN

It was first noticed in 2000 that S6K1-deficient mice were insulin deficient, probably owing to reduced β-cell mass (77). Later, George Thomas and colleagues (100) reported protection of S6K1-deficient mice against obesity-induced insulin resistance. Although S6K1-deficient mice display only slightly improved insulin sensitivity in comparison with wild types when fed chow, S6K1-deficient mice do not develop insulin resistance when fed HFD. Insulin binding to its receptor induces tyrosine phosphorylation of insulin receptor substrate (IRS) proteins. Signaling through mTOR can downregulate insulin signaling through phosphorylation by mTOR or S6K1 of IRS-11 on Ser307 (32, 103). mTOR is normally activated in response to insulin. IRS phosphorylation induces PI3K activation, leading to Akt phosphorylation. Akt phosphorylates and inhibits TSC1/2, activating Rheb and mTOR (3). Consequently, mTOR-induced IRS-1 desensitization acts as a negative feedback loop under normal circumstances. Overnutrition, however, can lead to insulin resistance via overabundant mTOR signaling. Indeed, mTOR signaling is increased in obese rat liver (98, 41) and muscle (41), two important insulin-responsive tissues.

Consistent with a role for activation of the mTOR pathway to attenuate insulin signaling, Thomas and colleagues report decreased phosphorylation of IRS1 at Ser307, Ser636, and Ser639 in S6K1-deficient mice, compared with wild-type, after four months on HFD (100). This effect is mimicked after rapamycin treatment, which increases insulin-stimulated phosphorylation of IRS1-associated PI3K activity and of Akt on Thr308 and Ser308 (41).

In addition to its important actions in peripheral tissues, insulin also acts in the brain to control glucose homeostasis. Normally, central insulin action suppresses hepatic glucose production (HGP), acting to reduce blood glucose levels. As it does in peripheral organs, mTOR signaling in the brain reduces insulin signaling. Infusion of adenoviral vector encoding constitutively active S6K1 into the MBH impairs the ability for intra-MBH insulin to induce Akt Thr308 and Ser473 phosphorylation and to suppress HGP (74). The ability for S6K1 to abolish this normal response to insulin is probably due to its action at IRS-1, whose phosphorylation was increased in MBH lysates from these rats. This mechanism appears also to explain central insensitivity to leptin during overfeeding. During HFD feeding, insulin fails to suppress

HGP. Infusion of an adenoviral vector encoding either a kinase-dead, dominant-negative form of S6K1 or a C-terminal-truncated raptor enhances insulin-induced IRS-1 and Akt phosphorylation and restores HGP in HFD-fed rats. These data support a link between S6K1 overactivation and insulin resistance in obesity.

CONCLUSION

From an understanding of the mechanisms for cellular fuel sensing have emerged hypotheses regarding the role of brain fuel sensing in maintaining energy homeostasis. Like peripheral cells, neurons are known to respond to glucose, fatty acids, and amino acids. Manipulation of these signals in key hypothalamic regions has been shown to modulate food intake behavior. The mechanisms by which these fuels are sensed and by which the behavioral changes are effected have yet to be elucidated, but a large body of data implicates that both AMPK and mTOR may be sensors linking signals of fuel availability to behavioral effector systems such as the melanocortin system.

Identification of the downstream targets to which central nutrient sensors project in order to control ingestive behavior is a goal that should be given significant priority. The data suggest that mTOR signaling is important in AgRP neurons, yet we cannot rule out a role for POMC neurons. Furthermore, mTOR appears to play an important role in mediating the effects of hormones known to interact both with AgRP and POMC neurons. Whether mTOR is a generic or a cell type–specific fuel sensor within the hypothalamus must be clarified by future studies.

Neuronal fuel sensing appears to serve as a nexus between nutrient and hormonal signaling in the hypothalamus and thereby provides a mechanism by which signals of available fuel can be integrated with the hormonal signals that reflect the levels of stored fuel. A key goal is to identify aspects of these fuel-sensing systems that are specific to the brain. Such brain-specific components of these fuel-sensing systems may become targets for pharmacological intervention. Maybe more importantly, however, our understanding of the signaling aspects of nutrients allows for designing diets by their signaling properties. Thus, carefully designed dietary interventions may provide less expensive and more widely available solutions to treating metabolic diseases including obesity. That is a future at which we desperately need to arrive sooner rather than later.

SUMMARY POINTS

1. Strong homeostatic regulation promotes hyperphagia in response to weight loss, making weight loss by dieting rarely effective.
2. Neuronal fuel monitoring adjusts food intake behavior in response to nutrient availability for the organism.
3. The mammalian target of rapamycin (mTOR) is a cellular fuel sensor that has been adapted as a key component of the energy balance regulatory system in the CNS.

FUTURE ISSUES

1. An important research focus should be the specific downstream mechanisms by which mTOR regulates food intake. A crucial aspect of this focus will be to distinguish those functions of mTOR that are for cellular growth and activation versus those adapted specifically for maintenance of whole-body energy homeostasis.

2. Various energy sources (glucose, lipids, and amino acids) interact to regulate mTOR in the CNS.
3. The relationship between mTOR and other fuel-sensing systems in the CNS needs to be determined.

DISCLOSURE STATEMENT

Dr. Seeley receives research support and consulting fees from several companies interested in treatments for obesity and diabetes. These include Johnson & Johnson, Eli Lilly, Amylin Pharmaceuticals, Novo Nordisk, Merck, and Zafgen.

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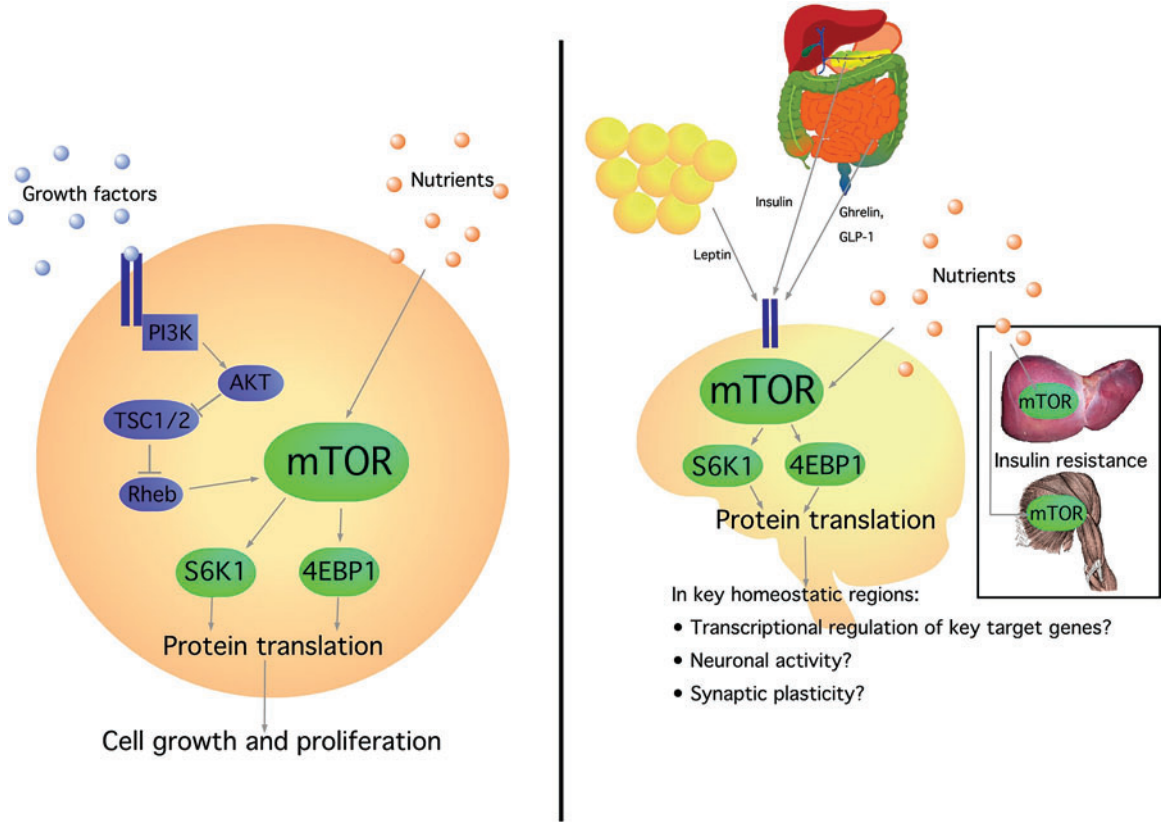

Figure 1

Central nervous system fuel-sensing mechanisms. mTOR, mammalian target of rapamycin.

Annual Review of Nutrition
Volume 30, 2010

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Errata

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