hemichannel makes it a major contributor to ionic dysregulation in ischemia. Second, Px1 hemichannel opening may result in efflux of glucose and adenosine triphosphate (ATP), further compromising the neuron's recovery from an ischemic insult. Consistent with this was our observation that fluorescent dyes became membrane-permeable only during OGD. Hemichannels are putative conduits for ATP release from astrocytes (*21*) and in the cochlea (*22*). Third, the large amplitude of the Px1 hemichannel current at holding potentials near the neuron's resting membrane potential (~ –60 mV) indicates that these currents likely contribute substantially to "anoxic depolarization," a poorly understood but well-recognized and key component of ischemic neuronal death (*2*, *23*, *24*). Therefore, hemichannel opening may be an important new pharmacological target to prevent neuronal death in stroke.

References and Notes

1. A. J. Hansen, *Physiol. Rev.* **65**, 101 (1985).
2. P. Lipton, *Physiol. Rev.* **79**, 1431 (1999).
3. M. Kamermans *et al.*, *Science* **292**, 1178 (2001).
4. J. E. Contreras *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 495 (2002).
5. R. P. Kondo, S. Y. Wang, S. A. John, J. N. Weiss, J. I. Goldhaber, *J. Mol. Cell. Cardiol.* **32**, 1859 (2000).
6. H. Li *et al.*, *J. Cell Biol.* **134**, 1019 (1996).
7. L. Bao, S. Locovei, G. Dahl, *FEBS Lett.* **572**, 65 (2004).
8. R. Bruzzone, M. T. Barbe, N. J. Jakob, H. Monyer, *J. Neurochem.* **92**, 1033 (2005).
9. R. Bruzzone, S. G. Hormuzdi, M. T. Barbe, A. Herb, H. Monyer, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13644 (2003).
10. See supporting material on *Science* Online.
11. J. Gao *et al.*, *Neuron* **48**, 635 (2005).
12. M. Aarts *et al.*, *Cell* **115**, 863 (2003).
13. C. Tomasetto, M. J. Neveu, J. Daley, P. K. Horan, R. Sager, *J. Cell Biol.* **122**, 157 (1993).
14. G. Feng *et al.*, *Neuron* **28**, 41 (2000).
15. A. Nimmerjahn, F. Kirchhoff, J. N. Kerr, F. Helmchen, *Nat. Methods* **1**, 31 (2004).
16. G. Sohl, S. Maxeiner, K. Willecke, *Nat. Rev. Neurosci.* **6**, 191 (2005).
17. J. C. Saez, M. A. Retamal, D. Basilio, F. F. Bukauskas, M. V. Bennett, *Biochim. Biophys. Acta* **1711**, 215 (2005).
18. R. J. Thompson, M. H. Nordeen, K. E. Howell, J. H. Caldwell, *Biophys. J.* **83**, 278 (2002).
19. M. L. Fung, G. G. Haddad, *Brain Res.* **762**, 97 (1997).
20. H. Benveniste, J. Drejer, A. Schousboe, N. H. Diemer, *J. Neurochem.* **43**, 1369 (1984).
21. C. E. Stout, J. L. Costantin, C. C. Naus, A. C. Charles, *J. Biol. Chem.* **277**, 10482 (2002).
22. H. B. Zhao, N. Yu, C. R. Fleming, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18724 (2005).
23. T. R. Anderson, C. R. Jarvis, A. J. Biedermann, C. Molnar, R. D. Andrew, *J. Neurophysiol.* **93**, 963 (2005).
24. G. G. Somjen, *Physiol. Rev.* **81**, 1065 (2001).

25. Supported by the Canadian Institutes for Health Research and the Canadian Stroke Network. B.A.M. has a Tier 1 Canada Research Chair in Neuroscience and a Michael Smith Foundation for Health Research distinguished scholar award. We thank Y.-T. Wang, C. C. Naus, and T. Snutch for critical reading of the manuscript.

Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5775/924/DC1
Materials and Methods

14 February 2006; accepted 31 March 2006
10.1126/science.1126241

Hypothalamic mTOR Signaling Regulates Food Intake

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The mammalian Target of Rapamycin (mTOR) protein is a serine-threonine kinase that regulates cell-cycle progression and growth by sensing changes in energy status. We demonstrated that mTOR signaling plays a role in the brain mechanisms that respond to nutrient availability, regulating energy balance. In the rat, mTOR signaling is controlled by energy status in specific regions of the hypothalamus and colocalizes with neuropeptide Y and proopiomelanocortin neurons in the arcuate nucleus. Central administration of leucine increases hypothalamic mTOR signaling and decreases food intake and body weight. The hormone leptin increases hypothalamic mTOR activity, and the inhibition of mTOR signaling blunts leptin's anorectic effect. Thus, mTOR is a cellular fuel sensor whose hypothalamic activity is directly tied to the regulation of energy intake.

A subset of neurons in the central nervous system (CNS) plays a role in regulating both blood plasma fuel levels and nutrient intake (*1*, *2*). An emerging concept is that specific neuronal populations integrate fuel availability signals with signals mediated by hormones such as leptin (*3*). However, the signaling pathways that are involved are poorly understood.

In peripheral cells, the mammalian mTOR signaling pathway integrates nutrient signals with hormonal signals to control growth and development (*4*, *5*). mTOR is a highly conserved serine-threonine kinase, which, in the presence of mitogens and available nutrients (including amino acids), stimulates protein synthesis and inhibits autophagy (*6*). In vitro, cellular levels of adenosine triphosphate (ATP) increase mTOR signaling, and mTOR itself is thought to serve as an ATP sensor (*7*). mTOR thus functions as a checkpoint by which cells sense and decode changes in energy status, which in turn determines the rate of cell growth and proliferation (*6*). Complete loss of TOR function is lethal in mice (*8*); in *Drosophila*, defects in TOR signaling result in the formation of smaller cells in all tissues (*9*). Conversely, increased or otherwise aberrant mTOR activity has been linked to the development of cancer, diabetes, and obesity (*10*, *11*). As is consistent with the development of these diseases, the activation of the mTOR pathway is markedly elevated in the liver and in the skeletal muscle of insulin-resistant obese rats maintained on a high-fat diet (*12*), whereas the absence of the downstream mTOR target [S6 kinase 1 (S6K1)] protects against diet-induced obesity and enhances insulin sensitivity in mice (*13*). Given these observations, we hypothesized that mTOR might integrate cellular fuel status with hormonal-related signaling in specific populations of neurons that use this information to regulate food intake.

To test this hypothesis, we used antibodies to localize mTOR and two downstream targets of mTOR action [S6K1 and S6 ribosomal protein (S6) (*5*, *6*)] in the rat brain. Consistent with previous work (*14*), antibodies recognizing total mTOR had a ubiquitous distribution in the CNS, and there was scattered expression of specific phosphorylation of mTOR at Ser^{2448} (pmTOR) in extra-hypothalamic areas, including the hippocampus, thalamus, and cortex. In the hypothalamus, pmTOR was highly localized in the paraventricular (PVN) and arcuate (ARC) nuclei (fig. S1A) (*15*). Likewise, total S6K1 stained broadly throughout the CNS, whereas the hypothalamic expression of the activated form of S6K1, phosphorylated at Thr^{389} (pS6K1), was also largely limited to the PVN and ARC. Further, dual labeling for pmTOR and pS6K1 revealed that they are localized in the same cells in both of these regions (fig. S1B). Although most of these cells appear to be neurons, some may be glia.

The ARC contains at least two populations of neurons that are linked to the regulation of energy balance and whose activity is regulated by leptin: (i) orexigenic neurons that express both neuropeptide Y (NPY) and agouti-related peptide (AgRP) and (ii) anorexigenic neurons that express proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). Both pmTOR and pS6K1 were found in ~90% of ARC NPY/AgRP neurons (Fig. 1A), whereas only 45% of ARC POMC/CART neurons revealed phosphorylation of these proteins (Fig. 1B).

We next investigated whether changes in the body's energy status modulate mTOR signaling

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in the brain. There was a notable decrease in both hypothalamic pS6K1 and S6 phosphorylated at Ser^{240} and Ser^{244} (pS6) in rats that were fasted for 48 hours as compared with rats that were re-fed for 3 hours (Fig. 2A), but no significant changes in protein phosphorylation were found in extra-hypothalamic areas, such as the cortex and hippocampus (fig. S2A). After a 48-hour fast, the number of hypothalamic cells expressing pmTOR and pS6K1 was significantly decreased in the ARC, whereas no significant changes were observed in the PVN (Fig. 2B and fig. S2B). Thus, mTOR activity in the ARC is low when available fuels are low and the organism is predisposed to consume more calories.

If mTOR signaling is linked to the regulation of energy balance in the CNS, then manipulations of mTOR activity in the hypothalamus would be predicted to alter food intake. In a variety of model systems, mTOR activity is sensitive to levels of branched-chain amino acids, especially L-leucine (*16*, *17*). If increased hypothalamic mTOR signaling suppresses food intake, then the administration of L-leucine in the vicinity of the ARC (*15*) should produce anorexia. Intracerebroventricular administration of L-leucine [1.1 μg in 2 μl of phosphate-buffered saline (PBS) into the third ventricle] to 24-hour fasted rats before the onset of the dark cycle caused a decrease in food intake that was apparent 4 hours after treatment and lasted for 24 hours (Fig. 3A). The L-leucine–induced anorexia was accompanied by significant weight loss (Fig. 3B). In separate experiments, L-leucine also decreased food intake during the light cycle (fig. S3, A and B).

Unlike L-leucine, the intracerebroventricular administration of L-valine, another branched-chain amino acid, did not potently stimulate mTOR signaling (*16*, *17*), nor did it affect food intake and body weight (fig. S3, C and D). If our hypothesis is correct, doses of L-leucine that suppress food intake should also increase mTOR activity in the hypothalamus. Consistent with this, 45 min after intracerebroventricular administration of L-leucine, levels of pS6K1 were significantly increased in the hypothalamus (fig. S3E) whereas no changes were observed in pS6 levels at that time point (pS6/S6 L-leucine, 94.8 ± 5.3% versus pS6/S6 PBS, 100 ± 3.3%, $P = 0.4$). L-leucine–induced anorexia was also accompanied by significantly reduced NPY mRNA levels in the ARC (NPY mRNA after L-leucine, 85.6 ± 4.3% versus NPY mRNA after PBS, 100 ± 5.0%, $P < 0.05$), suggesting that hypothalamic mTOR signaling is selectively linked to the NPY system. Moreover, control experiments revealed that the L-leucine–induced anorexia was not due to the development of conditioned taste aversions (fig. S3, F and G).

We next investigated the effects of the well-characterized mTOR inhibitor rapamycin (*18*). Intracerebroventricular administration of

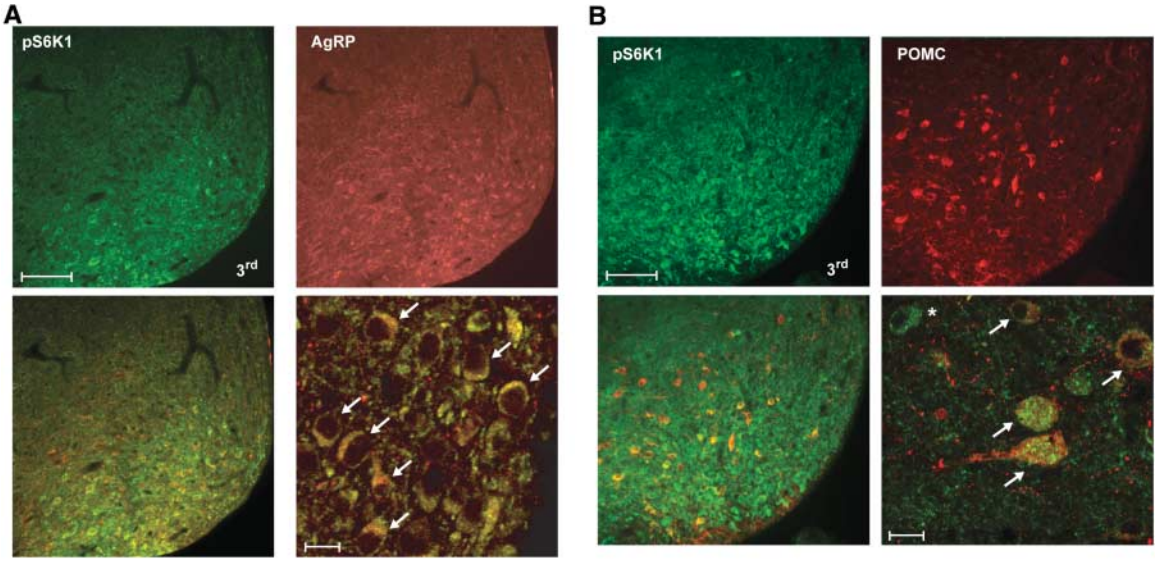

Fig. 1. Localization of mTOR signaling in the rat hypothalamus. (**A**) Immunohistochemistry showing colocalization (yellow, bottom left) of pS6K1 (green, top left) and AgRP (red, top right) in the ARC of the hypothalamus. Scale bar, 50 μm. (Bottom right) High-resolution image depicting the colocalization of pS6K1 and AgRP (arrows). 3rd indicates the third ventricle. Scale bar, 10 μm. (**B**) Immunohistochemistry showing colocalization (yellow, bottom left) of pS6K1 (green, top left) and POMC (red, top right) in the ARC of the hypothalamus. Scale bar, 50 μm. (Bottom right) High-resolution image depicting the colocalization of pS6K1 and POMC (arrows). The asterisk indicates a cell expressing only pS6K1. Scale bar, 10 μm.

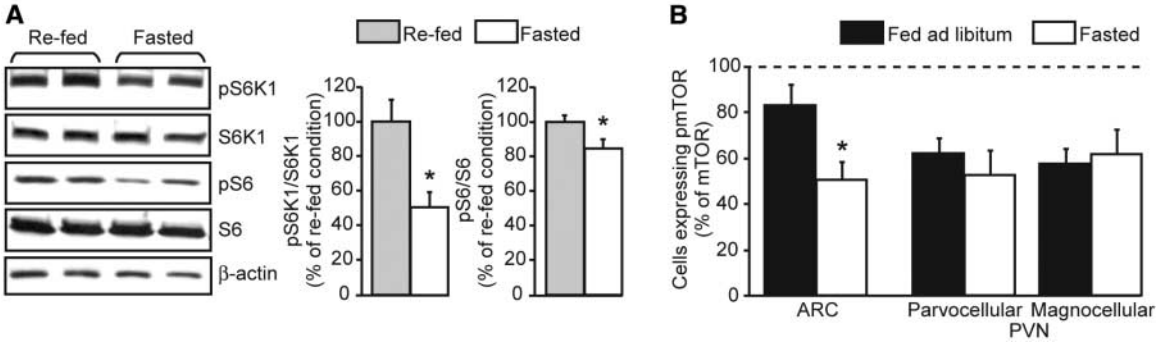

Fig. 2. Modulation of hypothalamic mTOR signaling by energy status. (**A**) (Left) Representative Western blot from re-fed rats or rats fasted for 48 hours. β-actin was the loading control. (Right) Quantification by image analysis of hypothalamic S6K1 and S6 phosphorylation. Error bars indicate SEM. *$P < 0.05$ versus rats in the re-fed condition. Five brains were examined for each condition. (**B**) Quantification of immunoreactivity for pmTOR in the ARC and PVN of rats fed ad libitum and rats fasted for 48 hours. The mean ± SEM of the number of cells positive for pmTOR is expressed as a percentage of mTOR-labeled cells. *$P < 0.05$ versus rats in the fed–ad libitum condition. Five brains were examined for each condition.

rapamycin [50 μg in 2 μl of dimethyl sulfoxide (DMSO)] rapidly inhibited hypothalamic S6K1 and S6 phosphorylation (Fig. 3C) and significantly increased the short-term intake of chow in pre-satiated rats, which were exposed to a highly palatable diet (Ensure) during the light cycle (fig. S4).

To determine whether the stimulation of hypothalamic mTOR signaling is required for the L-leucine–induced reduction of food intake, we combined intracerebroventricular administration of rapamycin, at a dose that does not affect food intake, with a subsequent intracerebroventricular injection of L-leucine. Rapamycin significantly inhibited the L-leucine–induced anorexia 4 hours after the administration of the amino acid (Fig. 3D), an effect that persisted up to 24 hours after treatment (Fig. 3D). Moreover, whereas L-leucine–treated rats lost a significant amount of weight, the rapamycin pre-treatment was associated with changes in body weight that were comparable to those observed in vehicle-treated animals (Fig. 3E).

A number of hormones and cytokines mediate their cellular effects through the mTOR signaling pathway. For example, the activation of mTOR and S6K1 by insulin is dependent on the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (*10*) and in the hypothalamus, the anorectic actions of both insulin and leptin can be blocked by the inhibition of PI3K (*19*, *20*). To determine whether leptin's anorectic effects depend on mTOR activation, we examined hypothalamuses from leptin-treated rats [10 μg in 2 μl of saline, intracerebroventricular (icv)] 2 hours after the administration of the hormone (Fig. 4A). Leptin treatment increased the phosphorylation of both S6K1 and S6 relative to saline. Moreover, a significant positive correlation was found between the hypothalamic phosphorylation levels of signal transducer and activator of transcription 3 (pSTAT3) and both pS6K1 (Pearson's $r = 0.6$, $P = 0.03$) and pS6 (Pearson's $r = 0.86$, $P = 0.0006$). To determine whether increased mTOR activity is required for the leptin-induced anorexia, we combined the administration of leptin with rapamycin. Rapamycin greatly attenuated the anorexia and body weight loss that was induced by leptin over a 24-hour period (Fig. 4, B and C). This contrasts with the results obtained with the potent melanocortin receptor 3 and 4 agonist, melanotan II (MTII) (0.26 μg in 1 μl of saline, icv), whose effect on food intake and body weight was unaffected by rapamycin (fig. S5, A and B). This finding suggests that the interaction between mTOR signaling and leptin is relatively specific.

Our data highlight an important role for hypothalamic mTOR signaling in food intake and energy balance regulation in a mammalian model. Analogous changes in S6K activity affect the feeding behavior of *Drosophila* larvae

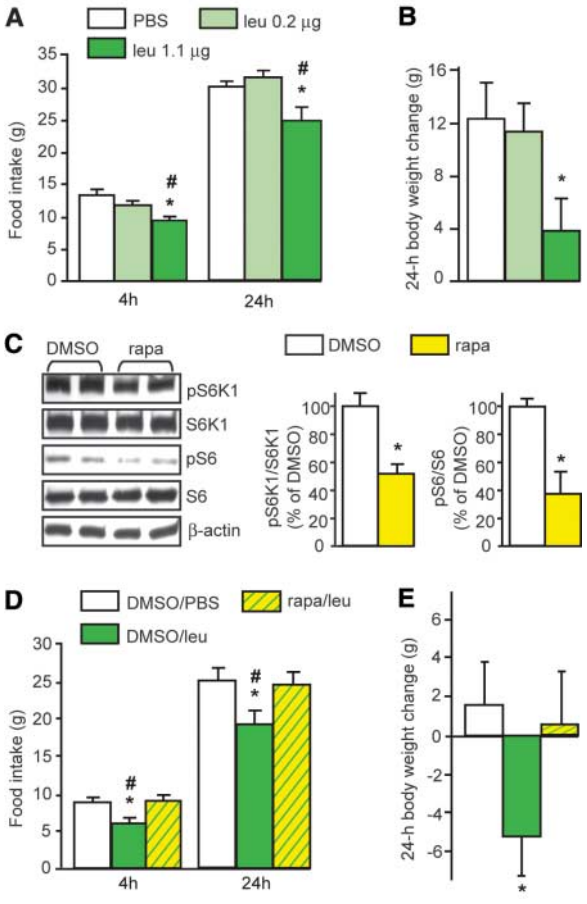

Fig. 3. L-leucine and rapamycin oppositely modulate hypothalamic mTOR signaling. (**A** and **B**) Intracerebroventricular administration of L-leucine decreases food intake (A) and body weight gain (B) in rats fasted for 24 hours. The mean ± SEM of 6 to 7 rats used for each treatment group is shown. *P < 0.05 versus PBS-treated rats; #P < 0.05 versus rats treated with 0.2 μg of L-leucine in 2 μl of PBS (leu 0.2). Leu 1.1, treatment with 1.1 μg of L-leucine in 2 μl of PBS. (**C**) Rapamycin (50 μg in 2 μl of DMSO, icv) inhibits hypothalamic mTOR signaling. (Left) Representative Western blot from DMSO- or rapamycin (rapa)–treated rats. β-actin was the loading control. (Right) Quantification by image analysis of hypothalamic S6K1 and S6 phosphorylation. Error bars indicate SEM. *P < 0.05 versus DMSO-treated rats. Three brains were examined for each condition. (**D** and **E**) Rapamycin (25 μg in 2 μl of DMSO, icv) blocks the L-leucine–induced effects on food intake (D) and body weight (E). The mean ± SEM of 6 to 7 rats used for each treatment group is shown. *P < 0.05 versus DMSO/PBS-treated rats; #P < 0.05 versus rapamycin/leucine-treated rats.

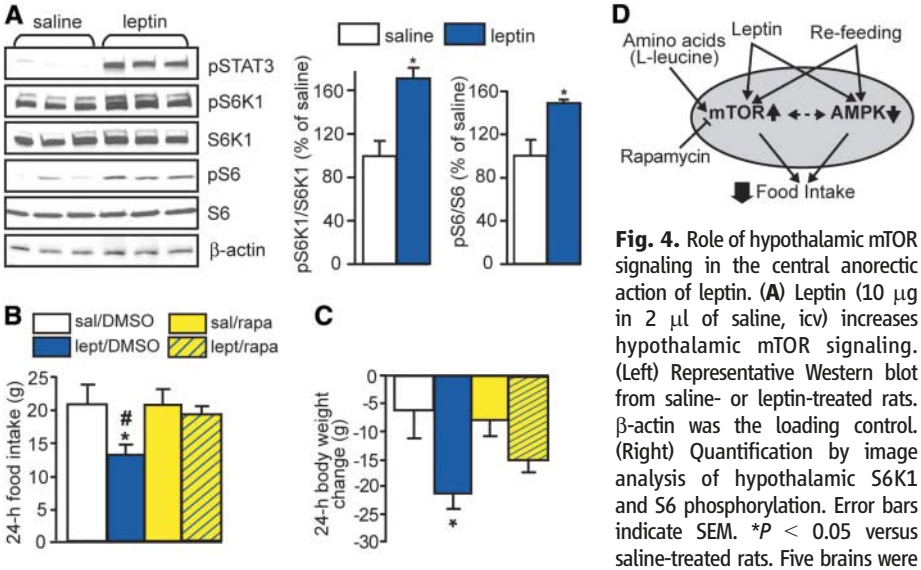

Fig. 4. Role of hypothalamic mTOR signaling in the central anorectic action of leptin. (**A**) Leptin (10 μg in 2 μl of saline, icv) increases hypothalamic mTOR signaling. (Left) Representative Western blot from saline- or leptin-treated rats. β-actin was the loading control. (Right) Quantification by image analysis of hypothalamic S6K1 and S6 phosphorylation. Error bars indicate SEM. *P < 0.05 versus saline-treated rats. Five brains were examined for each condition. (**B** and **C**) Rapamycin (25 μg in 2 μl of DMSO, icv) blocks the central action of leptin (10 μg in 2 μl of saline, icv) on food intake (B) and body weight changes (C). The data are shown as the mean ± SEM. Saline- or DMSO-treated rats ($n = 3$) were compared with treated rats ($n = 8$). *P < 0.05 versus saline/DMSO- or saline/rapamycin-treated rats; #P < 0.05 versus leptin/rapamycin-treated rats. (**D**) Proposed model for the role of mTOR signaling in the hypothalamic regulation of energy balance. Anorectic signals, such as amino acids (L-leucine), leptin, and re-feeding, increase hypothalamic mTOR signaling. Increased mTOR activity leads to a decrease in food intake. Rapamycin inhibits hypothalamic mTOR, causing an increase in food intake. Opposite to their effect on mTOR, leptin and re-feeding decrease hypothalamic AMPK (*27*). mTOR is inhibited by AMPK-dependent mechanisms in vitro (*28*). Thus, reciprocal interaction might exist between hypothalamic mTOR and AMPK.

(*21*). Cells suppress protein synthesis when there is insufficient energy or amino acid substrate, and mTOR plays a critical role in this regulatory mechanism. Similarly, the CNS must monitor fuel and substrate levels to coordinate the availability of fuel for the entire organism. Whereas a signal of low fuel in a single peripheral cell may curtail its own protein synthesis, causing a localized catabolic action, a signal of low fuel in key areas of the CNS might be expected to increase food intake, producing an overall anabolic effect.

CNS circuits can directly sense glucose and specific fatty acids, integrating this information to modulate caloric intake (*1*, *22*). Our findings expand the knowledge of these CNS sensing mechanisms to include a protein component: the amino acid L-leucine. However, the degree to which amino acids act as physiological signals to centrally modulate energy balance, in situations other than amino acid imbalance (*23*), is unclear. The ability of L-leucine to activate mTOR in the hypothalamus and to inhibit food intake may be an example of CNS circuits using an evolutionarily conserved signaling mechanism as a fuel sensor rather than as an amino acid sensor.

An important feature of the mTOR pathway is that its activity is modulated by growth factors and hormones. Our data indicate that hypothalamic mTOR signaling can be modulated by leptin and that leptin's effect on food intake is mTOR-dependent.

One implication of our experiments relates to recent findings describing leptin induced rapid reorganization of synapses in the ARC (*24*). Local regulation of mRNA translation plays an important role in axon guidance and neuronal plasticity, which are processes that involve mTOR activity (*25*). Conceivably, the inhibition of mTOR signaling may block leptin's anorectic effect by suppressing the hormone's synaptic remodeling activity.

Other fuel-sensitive kinases have been implicated in the hypothalamic control of energy balance. Like mTOR, AMP-activated protein kinase (AMPK) is regulated by intracellular AMP/ATP ratios. However, in contrast to mTOR, AMPK activity is increased during fuel deficiency (*26*) and inhibited by leptin and nutrient signals (*27*). AMPK overexpression in the hypothalamus increases food intake and body weight, and its down-regulation inhibits feeding (*27*). Moreover, the activation of AMPK-dependent mechanisms leads to the inhibition of mTOR activity (*28*). Thus, AMPK and mTOR may have overlapping and reciprocal functions (Fig. 4D).

These fuel-sensitive signaling pathways may ultimately provide important insights into the link between obesity and type 2 diabetes. In peripheral organs such as the liver, and in skeletal muscle, fuel overabundance is deleterious because it alters the activity of fuel-sensitive kinases (increased mTOR and decreased AMPK), causing insulin resistance, which in turn suppresses nutrient uptake into tissues (*10*, *26*). In the CNS, an overabundance of fuel and nutrients may produce similar changes in mTOR and AMPK signaling, leading, conversely, to a beneficial reduction in nutrient intake and in the level of stored fat. As long as the CNS responses are adequate, the organism can remain in a state of metabolic balance. However, an imbalance between peripheral and CNS fuel-sensing pathways may predispose toward the development of obesity and/or diabetes.

References and Notes

1. B. E. Levin, A. A. Dunn-Meynell, V. H. Routh, *Int. Rev. Neurobiol.* **51**, 219 (2002).
2. S. Ritter, K. Bugarith, T. T. Dinh, *J. Comp. Neurol.* **432**, 197 (2001).
3. R. J. Seeley, S. C. Woods, *Nat. Rev. Neurosci.* **4**, 901 (2003).
4. D. M. Sabatini, H. Erdjument-Bromage, M. Lui, P. Tempst, S. H. Snyder, *Cell* **78**, 35 (1994).
5. T. Schmelzle, M. N. Hall, *Cell* **103**, 253 (2000).
6. E. Jacinto, M. N. Hall, *Nat. Rev. Mol. Cell Biol.* **4**, 117 (2003).
7. P. B. Dennis *et al.*, *Science* **294**, 1102 (2001).
8. Y. G. Gangloff *et al.*, *Mol. Cell. Biol.* **24**, 9508 (2004).
9. J. Montagne *et al.*, *Science* **285**, 2126 (1999).
10. B. D. Manning, *J. Cell Biol.* **167**, 399 (2004).
11. K. Inoki, M. N. Corradetti, K. L. Guan, *Nat. Genet.* **37**, 19 (2005).
12. L. Khamzina, A. Veilleux, S. Bergeron, A. Marette, *Endocrinology* **146**, 1473 (2005).
13. S. H. Um *et al.*, *Nature* **431**, 200 (2004).
14. C. J. Sabers *et al.*, *J. Biol. Chem.* **270**, 815 (1995).
15. Materials and methods are available as supporting material on *Science* Online.
16. A. J. Meijer, P. F. Dubbelhuis, *Biochem. Biophys. Res. Commun.* **313**, 397 (2004).
17. C. G. Proud, *Eur. J. Biochem.* **269**, 5338 (2002).
18. M. A. Bjornsti, P. J. Houghton, *Nat. Rev. Cancer* **4**, 335 (2004).
19. K. D. Niswender *et al.*, *Nature* **413**, 794 (2001).
20. K. D. Niswender *et al.*, *Diabetes* **52**, 227 (2003).
21. Q. Wu, Y. Zhang, J. Xu, P. Shen, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13289 (2005).
22. T. K. Lam, G. J. Schwartz, L. Rossetti, *Nat. Neurosci.* **8**, 579 (2005).
23. D. W. Gietzen, L. J. Magrum, *J. Nutr.* **131**, 851S (2001).
24. S. Pinto *et al.*, *Science* **304**, 110 (2004).
25. G. M. Schratt, E. A. Nigh, W. G. Chen, L. Hu, M. E. Greenberg, *J. Neurosci.* **24**, 7366 (2004).
26. B. B. Kahn, T. Alquier, D. Carling, D. G. Hardie, *Cell. Metab.* **1**, 15 (2005).
27. Y. Minokoshi *et al.*, *Nature* **428**, 569 (2004).
28. K. Inoki, T. Zhu, K. L. Guan, *Cell* **115**, 577 (2003).

29. We thank L. De, E. Matter, K. Parks, J. Reed, E. Richer, D. Russell, J. Sorrell, and M. Toure for expert technical assistance; J. P. Herman and C. M. Padgett for assistance with artwork preparation; and H. Shi for assistance with the quantification of the immunohistochemistry. Supported by NIH grants DK 17844, DK 54080, and DK 54890. G.T. receives research support from Novartis.

Supporting Online Material

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21 December 2005; accepted 25 March 2006
10.1126/science.1124147