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"AMPing up" our understanding of the hypothalamic control of energy balance

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Commentary

AMP-activated protein kinase (AMPK) has emerged as a metabolic "fuel gauge," which oscillates between anabolic and catabolic processes that ultimately influence energy balance. A study in this issue of the *JCI* by Claret et al. now extends the role of AMPK in medial basal hypothalamic neurons (see the related article beginning on page 2325). These findings maintain AMPK signaling as a common cellular mechanism in proopiomelanocortin and neuropeptide Y/agouti-related protein neurons and links hypothalamic AMPK to coordinated energy and glucose homeostasis.

confirmation by vigorous scientific studies. The demonstration of autocrine/paracrine regulation of TRPM6 by EGF adds a new chapter in the journey toward this goal.

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"AMPing up" our understanding of the hypothalamic control of energy balance

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AMP-activated protein kinase (AMPK) has emerged as a metabolic "fuel gauge," which oscillates between anabolic and catabolic processes that ultimately influence energy balance. A study in this issue of the *JCI* by Claret et al. now extends the role of AMPK in medial basal hypothalamic neurons (see the related article beginning on page 2325). These findings maintain AMPK signaling as a common cellular mechanism in proopiomelanocortin and neuropeptide Y/agouti-related protein neurons and links hypothalamic AMPK to coordinated energy and glucose homeostasis.

As we live in the midst of rising rates of obesity, diabetes, and associated comorbidities, intense interest exists in increasing the understanding of the cellular and molecular mechanisms by which nutrients and metabolic cues modulate neuronal activity and how neurons may ultimately regulate energy homeostasis. Key targets of such cues are neurons that reside in the medial basal hypothalamus. The prototypical "sensing" cells are proopiomelanocortin (POMC) and neuropeptide Y/agouti-related protein (NPY/AgRP) neurons in the arcuate nucleus of the hypothalamus. A wealth of data has demonstrated the inherent ability of these neurons to respond to changing levels of a number of signals including insulin, leptin, and glucose. The ability of these (and other) neurons to sense and integrate coordinated responses to changing levels of metabolic signals is thought to contribute to the control of energy balance (1–5). On the other hand, it is becoming apparent that dysregulation of this regulatory system contributes to the pathophysiology of obesity, diabetes, and other components of the metabolic syndrome (6–8).

In addition to identifying the key sensing neurons, we now are beginning to

Nonstandard abbreviations used: AgRP, agouti-related protein; AMPK, AMP-activated protein kinase; NPY, neuropeptide Y; POMC, proopiomelanocortin.

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understand the signaling pathways that mediate these effects within respective cell types. For example, it has been suggested that the JAK/STAT, PI3K, and mammalian target of rapamycin (mTOR) pathways contribute to the actions of leptin in hypothalamic neurons (8–11). In addition, the 5′ AMP-activated protein kinase (AMPK) pathway has been identified as a key molecular signaling pathway in the coordinated control of energy balance (12). This is due in large part to the ability of the enzyme to link changes in the AMP/ATP ratio to coordinated cellular responses. AMPK regulates a vast array of processes in various tissues that appear to coordinate a "switch" between anabolic (energy consuming) and catabolic (energy producing) activities in various metabolically active tissues (reviewed in refs. 13, 14). Briefly, an acute rise in the AMP/ATP ratio, as occurs during single bouts of exercise, results in transient activation of AMPK and downstream catabolic pathways. Moreover, AMPK appears to be sensitive to changing levels of metabolic cues, including leptin, insulin, and nutrients. Increases in AMPK activity contribute to fatty acid oxidation and increased glucose transport concomitant with insertion of glucose transporter 4 (GLUT4) into the plasma membrane of muscle (15, 16). Another recent *JCI* article, by Tian et al., also suggests that AMPK is a key regulator of glycogen metabolism in cardiomyocytes (17). Moreover, AMPK activation leads to decreased hepatic glucose production and lipid synthesis but increased lipid oxidation in the liver and decreased glucose-dependent insulin secretion in pancreatic islet β cells (14). The ability of AMPK to detect cellular energy needs in order to trigger either anabolic or catabolic processes throughout the body has led several groups to suggest that AMPK is a metabolic "energy gauge/fuel sensor" important for coordinated energy homeostasis.

In addition to these actions in peripheral tissues, recent advances have identified potential regulators of AMPK activity in the brain. Contrary to reports on AMPK in muscle, several reports suggested that the anorexigenic signal leptin negatively regulates AMPK activity in the hypothalamus (18). Moreover, a decrease in hypothalamic AMPK activity is sufficient to reduce food intake and weight gain, while constitutive AMPK activation leads to hyperphagia and obesity (14, 18). However, the identity of the specific neurons in which AMPK mediates effects on energy balance has proven elusive.

AMPK in melanocortin neurons regulates energy balance

In the current issue of the *JCI*, Claret and colleagues have used the power of mouse genetics to directly investigate the physiological role of AMPK in POMC and NPY/AgRP neurons (19). Specifically, the authors generated mice lacking AMPKα*2* specifically in POMC- or AgRP-expressing neurons (POMCα*2*KO or AgRPα*2*KO mice, respectively) and used multiple parallel approaches to study the effects of these manipulations on long-term body weight and acute responses to changing levels of leptin, insulin, and glucose. In accordance with the model predicted by Minokoshi and colleagues (18), Claret et al. found that deletion of AMPK in AgRP neurons reduced body weight. In addition, they found that selective deletion of AMPKα2 in POMC neurons resulted in increased body weight and adiposity — an effect that seemed to be due to reduced energy expenditure (19). Collectively, these data establish that AMPK signaling in POMC and AgRP cells is necessary for proper long-term energy balance.

However, as is often the case in complex genetic studies, several unexpected results were also uncovered. For example, leptin and insulin are required for proper energy balance (1–5). However, deletion of AMPKα2 in POMC and AgRP neurons did not abolish the acute effects of leptin and insulin in these neurons (19). Specifically, the authors used patch-clamp electrophysiology techniques and determined that the regulatory effects of leptin and insulin on acute cellular responses were unaltered in POMCα2KO and AgRPα2KO neurons. In addition, the baseline biophysical properties of these neurons appeared to be intact. Thus, this manipulation resulted in changes in body weight, while the acute cellular responses to leptin and insulin remained intact. As noted, leptin and insulin are known to activate various intracellular signaling cascades in neurons. Thus, it is likely that the acute leptin-induced modulation of melanocortin neuronal activity does not require AMPK. However, longer-term effects of leptin action on these neurons are less clear. It will be important to further investigate the role AMPK may play in the long-term effects of leptin in this interesting model. Moreover, it is always important to note that the effects of leptin on food intake and body weight are mediated only in part by direct actions on POMC and AgRP neurons (6). It is likely that the regulatory effects of leptin on food intake and body weight are mediated by a distributed network of leptin-responsive cells on which AMPK may play a role (20–22).

AMPK: a molecular player in neuronal glucose sensing

While glucose is a universal fuel, changes in glucose levels also alter the firing rate of several hypothalamic neurons including POMC and NPY/AgRP neurons that may be AMPK dependent (23–25). Thus, Claret and colleagues investigated the effect of selective deletion of AMPK on glucose sensing in these cells (19). They found that selective deletion of AMPKα2 blunted the ability of POMC and AgRP neurons to respond to changing levels of glucose. As in the leptin and insulin studies, the authors used patch-clamp electrophysiology techniques to assess the acute responses to various concentrations of glucose. The firing rates of POMCα2KO or AgRPα2KO neurons were not reduced, as they were in the intact control neurons, when extracellular glucose levels were changed from 2 mM to 0.1 mM. These results suggest that AMPK activity in hypothalamic neurons is a link between hypoglycemia and cellular activity. The authors of the current study also reported that POMC neurons were not activated by rising glucose levels (19). This is surprising, as Ibrahim et al. demonstrated that POMC neurons are in fact glucose excited (26). It is known that the ATP-dependent closure of ATP-activated K^+ channels is required for glucose effects in this type of neuron, and therefore intracellular ATP levels must remain plastic. The reason for the discrepancy between the current study and that of Ibrahim et al. remains unclear, but future studies will likely focus on inherent technical issues that may explain the different results. Moreover, Claret et al. observed a similar excitability in AgRP cells. This is also unexpected, since recent reports have suggested opposing effects of glucose in POMC and NPY/AgRP neurons (1, 26–28). Despite the noted differences from previous studies, the current results (19) raise the fascinating possibility that glucose-activated and glucose-inhibited neurons may use a common cellular glucose-sensing mechanism. In this scenario, neurons

such as the POMC neuron may use molecular mechanisms including ATP-activated K^+ channels to link increased glucose levels to neuronal activity. In parallel, these cells may utilize AMPK-dependent mechanisms when glucose levels drop below euglycemia. Additional studies seem required to assess the role of AMPK in sensing rising glucose levels.

The physiological role and the context in which glucose sensing in the brain is important are still not well understood (24, 25, 29). One hypothesis is that it serves to slow down neuronal activity when glucose levels are low, thus preventing neuronal damage in these conditions. However, this neuroprotective adaptation would take place only in a small fraction of neurons. Indeed, in the majority of them, there would be either an increase or no change in activity under such conditions. Glucose sensing may be alternatively seen as an ancient mechanism used in single-cell or simple organisms to adjust their functions during conditions of food scarcity that has evolved to be part of pathways coordinating counterregulatory responses to hypoglycemia in more complex organisms, including mammals. It is of interest that an ancient metabolic-sensor protein such as AMPK was found by Claret et al. (19) to be required for neuronal glucose sensing in response to falling glucose levels.

Future directions: a need for multifaceted approaches

Not surprisingly, the current results raise several questions for future investigation. For instance, since AMPK is not an ion channel, how does it link reduced glucose to membrane potential? Does it phosphorylate an ion channel? Also, do POMCα2KO or AgRPα2KO mice have impaired counterregulation when challenged with severe hypoglycemia? These questions are intriguing to consider in light of the fact that altered counterregulation is a serious (even lethal) side effect of intense pharmacological treatments against diabetes and may be caused by dysfunctions in neuronal glucose-sensing mechanisms. Further, AMPK has been suggested to modulate several conductances in pancreatic β cells (30). It will be of interest to see whether AMPK influences a similar conductance in the brain (31).

Finally, the report by Claret and colleagues is an excellent illustration of the need to directly test current models of body weight and glucose homeostasis by making specific modifications in an otherwise intact in vivo context (19). Given the inherent complexity of the hypothalamus, these studies also nicely demonstrate the need for multidisciplinary, parallel, and complementary in vivo and in vitro approaches while using these models. These types of studies are inherently difficult, and researchers face several limitations, including lack of specific reagents (e.g., neuron-specific Cre lines) and cellular redundancy, not to mention the time and cost involved to perform these complicated studies. However, studies such as those of Claret and colleagues often unearth unexpected findings that challenge conventional models. This type of observation would not result if single approaches were used to study the complexity underlying coordinated control of body weight and glucose homeostasis. Thus, as the field moves forward, new research will undoubtedly uncover unexpected results such as those of Claret et al., all of which will be needed to combat the growing problems that are obesity and diabetes.

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New TB vaccines: is there a requirement for CD8+ T cells?

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MHC class I–restricted CD8+ T cells are necessary to mount an immune response against *Mycobacterium tuberculosis*. *M. tuberculosis* antigens can enter MHC class I cross-processing pathways through a number of different mechanisms, including via the uptake of antigen-containing apoptotic vesicles released by infected cells. A study in this issue of the *JCI* by Hinchey and colleagues shows that *M. tuberculosis* inhibits host cell apoptosis and thus may interfere with optimal cross-priming and action of CD8+ T cells (see the related article beginning on page 2279). *M. tuberculosis* genetically modified to induce apoptosis is shown to be more effective in priming CD8+ T cells in vivo and therefore may be a more effective vaccine against tuberculosis than the currently utilized *M. bovis* BCG vaccine.

Mycobacterium tuberculosis continues to cause widespread morbidity and mortality in children and adults worldwide, despite the availability of relatively simple diagnostic tools, inexpensive and effective drugs, and public health infrastructures in most countries for control and treatment of tuberculosis (TB) (1). In adolescents and adults, TB is primarily caused by reactivation of latent/persistent *M. tuberculosis* bacilli and progression to active pulmonary disease. *M. bovis* bacille Calmette-Guérin (BCG), widely used as TB vaccine for newborns and effective in preventing disseminated *M. tuberculosis* disease in young children, is unable to prevent pulmonary (reactivation) TB in adolescents and adults (2, 3). The latter finding was reconfirmed in a recent study of BCG revaccination of more than 15,000 7- to 14-year-old school children in Brazil (4). Thus, an effective vaccine for the prevention of pulmonary TB in adolescents and adults, many of whom are latently infected with *M. tuberculosis* in countries in which TB is endemic, is urgently needed to control the TB pandemic.

Macrophage apoptosis and *M. tuberculosis*

During the last 20 years, great progress has been made in areas essential for new TB vaccine development, including mycobacterial genetics, TB immunology, and animal models of *M. tuberculosis* infection. Completion of the *M. tuberculosis* genome sequence combined with genetic tools to delete, add back, or complement mycobacterial genes allows one to determine the *M. tuberculosis* genes essential for survival in macrophages and animal models and those genes involved in resisting host immune responses (5, 6). *M. tuberculosis* readily infects macrophages, and macrophage apoptosis has developed as one host defense mechanism against infection. However, virulent *M. tuberculosis* has evolved to be capable of inhibiting macrophage apoptosis. The study by Hinchey et al. in this issue of the *JCI* (7) represents an elegant example of a combination of approaches from the 3 areas of research described above to determine the role of mycobacterial genes *secA2* and *sodA* in resisting macrophage apoptosis and to determine whether enhanced apoptosis of *secA2* gene–deleted *M. tuberculosis* (Δ*secA2*) is associated with increased cross-presentation of antigens to CD8+ T cells and improved immunity against an aerosol challenge with *M. tuberculosis* in vivo (7). Earlier studies established that SecA2 was required for secretion of superoxide dismutase A (SodA) by *M. tuberculosis* and that knocking out *secA* resulted in a less virulent organism (8). Superoxide anions can kill mycobacteria directly and induce macrophage apoptosis. Apoptosis kills intracellular mycobacteria by a superoxide-independent mechanism. Hinchey et al. (7) now show that, in vitro, a Δ*secA2* mutant causes increased caspase expression and macrophage apoptosis compared with WT *M. tuberculosis*. When extracellular SodA expression was restored in the Δ*secA2* mutant by adding an N-terminal signal sequence to *sodA*, the level of macrophage apoptosis were reduced to that observed in response to WT *M. tuberculosis*. Thus a link between SecA2-dependent SodA secretion and inhibition of macrophage apoptosis was established.

Cross-processing of *M. tuberculosis* for CD8+ T cells

Adaptive immunity mediated by T cells and the cytokines they secrete is essential for controlling initial *M. tuberculosis* infection (usually in the lungs) and preventing reactivation of latent/persistent *M. tuberculosis* bacilli residing in granulomas. T cell failure induced by malnutrition, aging, HIV-1 infection, or immune-suppressive drugs allows latent infection to progress to active TB. Multiple T cell subsets are activated by *M. tuberculosis* antigens, including MHC class II–restricted CD4+ and MHC class I–restricted CD8+ T cells, as well as γδ TCR+ T cells, CD1-restricted T cells, CD25+CD4+

Nonstandard abbreviations used: BCG, bacille Calmette-Guérin; Δ*secA2*, *secA2* gene–deleted *Mycobacterium tuberculosis*; SodA, superoxide dismutase A; TB, tuberculosis.

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