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Acute Glucose Response Properties Beyond Feeding

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Hypothalamic AgRP neurons potently coordinate feeding behavior to ensure an organism's viability. However, their acute role in glucose-regulatory function remains to be addressed. Steculorum *et al.* now report that activation of a specific set of AgRP neurons results in an impairment of insulin-stimulated glucose uptake in brown fat through a myogenic signature program.

Obesity is one of the most complicated medical epidemics currently affecting the Western world. This condition is caused by a complicated interplay of various factors, including altered feeding patterns, decreased energy expenditure, and altered glucose homeostasis. Many of these symptoms fall under the control of neural circuits that maintain homeostatic equilibrium. For example, agouti-related peptide (AgRP)-expressing and pro-opiomelanocortin (POMC) neurons localized in the arcuate nucleus of the hypothalamus (ARC) serve as a key substrate in feeding behavior, both necessary and sufficient to regulate appropriate energy levels (Aponte *et al.*, 2011; Gropp *et al.*, 2005; Krashes *et al.*, 2011; Yaswen *et al.*, 1999). Furthermore, chronic manipulations of these explicit cell-types directly affect glucose homeostasis (Hill *et al.*, 2010; Konner *et al.*, 2007). To date, however, no studies have investigated the capacity of these neurons to acutely control glucose or insulin homeostasis, thus obscuring direct mechanistic analyses and the putative neural circuitry involved.

In a new study by Brüning and colleagues (Steculorum, 2016) published in *Cell*, AgRP neurons are shown to directly affect insulin sensitivity through perturbation of insulin-stimulated glucose uptake in brown adipose tissue (BAT) within minutes to hours. The researchers utilized molecularly-directed chemogenetic and optogenetic techniques to stimulate the activity of either anabolic AgRP, or catabolic POMC neurons, and analyzed insulin sensitivity in an acute fashion. Remarkably, activation of AgRP neurons (but not POMC) rapidly and robustly impaired both glucose and insulin tolerance in the absence of

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food, exposing a critical regulatory role of AgRP neurons in modulating peripheral glucose accumulation during conditions of low energy resources.

To test the contribution of AgRP neurons on acute insulin resistance and delineate the peripheral system modulating this response, the researchers performed euglycemic/hyperinsulinemic clamps in mice whereby blood glucose levels are controlled at an equilibrium point after acutely raising blood insulin. Compared with littermate controls, mice with AgRP neurons active during this clamp demonstrated a lower glucose infusion rate necessary to maintain constant blood glucose. This suggested that glucose was not readily cleared from the bloodstream, and was elevated after AgRP stimulation. Interestingly, this did not impact hepatic glucose production; instead insulin-mediated glucose uptake into BAT was drastically diminished, without affecting white adipose tissue or skeletal muscle. The authors found elevated serum insulin, but not glucagon or corticosterone levels after acute stimulation, further substantiating the capability of AgRP neurons to directly impair systemic insulin sensitivity. This link to altered glucose uptake in BAT is strengthened by similar observations made in obese mice fed a high-fat diet, which is known to impact AgRP activity properties (Baver et al., 2014; Belgardt and Bruning, 2010).

If AgRP neuron activity somehow alters insulin sensitivity, particularly BAT's ability to take up glucose, BAT itself may undergo acute genetic expression changes upon activation of AgRP neurons. To investigate this, the researchers next looked for changes in global gene expression in BAT after AgRP neuron activation using a microarray-based approach. The most robustly upregulated RNA products were transcripts normally produced in muscle tissue including myostatin (*Mstn*); this upregulation seemed to depend on feeding state, as fasted mice also displayed an upregulation of *Mstn* and other muscle-associated transcripts. Exposure to myostatin inhibits differentiation of BAT progenitor cells into brown adipocytes (Kim et al., 2012), but β -adrenergic stimulation directs these progenitor cells towards differentiation into adipocytes. The likely source of this stimulation is sympathetic nerve activity (SNA), which may decrease in response to AgRP activity. Thus, the investigators directly recorded SNA in response to AgRP stimulation in anesthetized animals using sharp electrodes to record from isolated sympathetic nerves innervating BAT; indeed, this activation reduced sympathetic nerve tone, presumably lowering β -adrenergic stimulation and permitting upregulation of *Mstn*. According to *ex vivo* cell culture experiments, myostatin itself was revealed to decrease the ability of BAT to secrete insulin. To test whether AgRP activity impacted insulin sensitivity via β -adrenergic stimulation and myostatin induction, the authors promoted β -adrenergic stimulation and, in a separate series of experiments, blocked myosin activity using an antibody treatment. Indeed, insulin tolerance tests indicated that pretreatment with a β 3-agonist or an anti-myostatin antibody blocked the activity of AgRP on insulin sensitivity.

These experiments address the mechanism of AgRP-mediated systemic insulin sensitivity, but the neural circuitry relaying AgRP signaling to the sympathetic nervous system remained unexplored. Thus, the researchers manipulated specific AgRP projections to determine which downstream regions mediated the insulin sensitivity change observed following whole-population cell body activation, which nonspecifically provokes responses at all efferent targets. Selective stimulation of AgRP neuron terminals in the anterior region

of the ventrolateral bed nucleus of the stria terminalis (aBNSTvl), but not the dorsal medial aBNST, paraventricular hypothalamus, dorsal raphe or dorsal vagal complex, acutely modified insulin sensitivity through marked induction of *Mstn* transcriptional levels in BAT. Interestingly, while stimulation of AgRP projections to the lateral hypothalamus also altered insulin sensitivity, this manipulation failed to change *Mstn* expression in BAT.

Overall, Stecclorum and colleagues have demonstrated that AgRP neurons exert more complicated effects on energy balance than previously understood. These results suggest that AgRP-mediated insulin resistance arises quickly and independently of its melanocortin receptor-mediated feeding effects. Specifically, AgRP activity poly-synaptically suppresses SNA, acutely influencing the genetic composition of brown adipocytes to produce more muscle-related products, especially myostatin. These exciting findings advocate obesity interventions targeting multiple non-overlapping signaling systems for an optimal therapeutic benefit. Further studies mapping out the entire wiring diagram from AgRP→aBNSTvl→BAT will provide even more pharmacological targeting opportunities in combating diabetes and obesity. Clearly, this study will provide a strong bedrock for future investigations into the interplay between the nervous and endocrine systems.

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Figure 1. Acute Effects of AgRP Neuronal Stimulation on Insulin Sensitivity in the Mouse
Chemogenetic or optogenetic stimulation of AgRP neurons, including their projections to the anterior bed nucleus of the stria terminalis, ventrolateral portion (aBNSTvl) induces changes in blood glucose occurring within 30 minutes of stimulation onset. AgRP activity lowers glucose uptake by brown adipose tissue (BAT), rendering these tissues more insulin resistant. AgRP neurons induce this change by suppressing sympathetic nerve tone (SNA) onto BAT via β -adrenergic-dependent mechanisms. This reduced tone is sufficient to alter gene expression programs within brown adipocytes to a more myocyte-like expression profile. Importantly, expression of the myocyte-related gene myostatin, is directly linked to acutely suppressing insulin signaling and raising insulin resistance in brown adipocytes. NE (norepinephrine).