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Review

GABAergic signaling by AgRP neurons prevents anorexia via a melanocortin-independent mechanism

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ABSTRACT

The hypothalamic arcuate nucleus contains two anatomically and functionally distinct populations of neurons—the agouti-related peptide (AgRP)- and pro-opiomelanocortin (POMC)-expressing neurons that integrate various nutritional, hormonal, and neuronal signals to regulate food intake and energy expenditure, and thereby help achieve energy homeostasis. AgRP neurons, also co-release neuropeptide Y (NPY) and γ-aminobutyric acid (GABA) to promote feeding and inhibit metabolism through at least three possible mechanisms: (1) suppression of the melanocortin signaling system through competitive binding of AgRP with the melanocortin 4 receptors; (2) NPY-mediated inhibition of post-synaptic neurons that reside in hypothalamic nuclei; (3) GABAergic inhibition of POMC neurons in their post-synaptic targets including the parabrachial nucleus (PBN), a brainstem structure that relays gustatory and visceral sensory information. Acute ablation of AgRP neurons in adult mice by the action of diphtheria toxin (DT) results in precipitous reduction of food intake, and eventually leads to starvation within 6 days of DT treatment. Chronic delivery of bretazenil, a $GABA_A$ receptor partial agonist, into the PBN is sufficient to restore feeding and body weight when AgRP neurons are ablated, whereas chronic blockade of melanocortin 4 receptor signaling is inadequate. This review summarizes the physiological roles of a neural circuitry regulated by AgRP neurons in control of feeding behavior with particular emphasis of the GABA output to the parabrachial nucleus. We also describe a compensatory mechanism that is gradually engaged after ablation of AgRP neurons that allows mice to continue eating without them.

Contents

1. Arcuate neurons help regulate energy balance

The mammalian central nervous system has evolved complex mechanisms to maintain body weight and fat content at a relatively constant level over life-long period to cope with fluctuations in hormonal state, food supply, as well as changing environment. Adaptive modifications of eating behavior and energy expenditure

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promote survival; malfunction of these adaptive systems may underlie severe conditions such as anorexia nervosa and obesity (Pinel et al., 2000; Stricker and Woods, 2004). Following the discovery of leptin (Zhang et al., 1994), a satiety signal released from adipocytes, enormous progress has been made defining and characterizing hypothalamic neural circuitry that mediates food intake and energy balance by responding to a variety of peripheral hormonal and neuromodulatory signals (Elmquist et al., 1999; Morton et al., 2006; Schwartz et al., 2000). Meanwhile, gustatory and gastrointestinal signals, as well as cognitive centers regulating hedonic and reward responses are thought to impinge onto this homeostatic system to initiate proper motor activity toward either food-seeking behavior or satiation response (Abizaid et al., 2006; Coll, 2007; Grill, 2006; Kelley et al., 2005; Saper et al., 2002). The arcuate nucleus of the hypothalamus received the greatest attention because of its unique anatomical location where blood-borne signaling molecules, such as leptin and insulin, can readily penetrate local blood-brain-barrier, thus allowing the arcuate neurons to detect changes of peripheral metabolic state (Fry et al., 2007).

Two distinct populations of neurons in the arcuate, AgRP-expressing and nearby POMC-expressing neurons are thought to play prominent roles in integration of peripheral and central signals to modulate appetite and metabolism (Cone, 2005; Morton et al., 2006; Saper et al., 2002). POMC neurons display considerable heterogeneity by co-releasing cocaine and amphetamine regulated transcript (CART), GABA, and/or glutamate, presumably in a segregated pattern (Broberger et al., 1998; Collin et al., 2003; Cowley et al., 2001; Hentges et al., 2004, 2009; Horvath et al., 1997; Meister, 2007; Ovesjo et al., 2001). Evidence from immuno-colocalization and in vitro neuronal recording studies suggest that ~40% POMC neurons are GABAergic, while another ~25% exhibit glutamatergic characters (Hentges et al., 2004, 2009), raising the possibility that physiological divergence of POMC neurons in control of energy homeostasis may be regulated by discrete glutamatergic and GABAergic projections to downstream targets. The majority of AgRP neurons also produce NPY and GABA. We refer them as AgRP neurons because that is the signature molecule that is only expressed in these cells, whereas NPY and GABA are expressed widely in the brain. The AgRP neuron population appears to be simpler because targeted ablation of AgRP neurons in adult mice simultaneously destroys more than 95% of neuropeptide Y-expressing and ~60% of the GABAergic cells neurons in the arcuate (Luquet et al., 2005; Wu et al., 2008a). It is not yet clear whether distinct populations of AgRP neurons project to specific brain nuclei or whether all AgRP neurons send collaterals to all target nuclei.

2. AgRP neurons are essential for feeding by adult mice

Over the last two decades, a significant amount of research has been devoted to establishing the physiological role of neuropeptides and monoamine transmitters that act on various G protein-coupled receptors to modulate feeding behavior, whereas relatively little attention has focused on the role of GABA and glutamate—neurotransmitters that activate ion channels and account for the regulation of most synaptic activity—on feeding behavior (Cone, 2005; Meister, 2007; Morton et al., 2006; Saper et al., 2002; van den Pol, 2003). Genetic, pharmacological, and physiological studies collectively suggest that POMC neurons inhibit feeding while stimulating energy expenditure by releasing α-melanocyte stimulating hormone (α-MSH) and subsequently activating $G_{\alpha s}$-coupled melanocortin 3 or 4 receptors (MC3R and MC4R) on post-synaptic cells in the pariventricular nucleus and other brain regions (Cone, 2005). For instance, food restriction or leptin deficiency ($Lep^{ob/ob}$) reduces hypothalamic POMC mRNA expression, whereas its expression level increases in overfed rats (Hagan et al., 1999; Mizuno et al., 1998). MC_4R agonists reduce food intake in rodents, while MC_4R antagonists elicit a hyperphagic phenotype (Benoit et al., 2000; Fan et al., 1997). In knockout mouse models, obesity results from inactivation of genes coding for POMC, MC_3R, or MC_4R (Butler et al., 2000; Huszar et al., 1997; Yaswen et al., 1999). Mice lacking both *Mc3r* and *Mc4r* genes are more obese than either alone (Butler et al., 2000; Chen et al., 2000).

Whereas pharmacological or genetic manipulation of α-MSH signaling has dramatic effects on feeding and energy balance, the effects of manipulating the peptide signaling by AgRP neurons have been inconsistent. The orexigenic effect of NPY (or the related peptide YY) injected intracranially is well established (Kalra et al., 1999) and chronic treatment with NPY leads to obesity (Beck et al., 1992; Raposinho et al., 2001). However, mice that chronically over-express neuropeptide Y by ~4-fold from the endogenous *Npy* locus have normal body weight (Ste Marie et al., 2005). Some NPY receptor antagonists suppress feeding, at least transiently (Beck, 2006; Billington et al., 1991; Egawa et al., 1991; Kalra et al., 1999; Stanley et al., 1986; Zarjevski et al., 1993), but inactivation of the *Npy* gene has a negligible effect on feeding or body weight regulation (Erickson et al., 1996; Palmiter et al., 1998). Genetic inactivation of genes encoding neuropeptide Y1 or Y5 receptors, also fails to inhibit feeding, and may even lead to late-stage obesity (Marsh et al., 1998; Pedrazzini et al., 1998; Qian et al., 2002; Thorsell and Heilig, 2002). Likewise, intracranial injection of AgRP has a long-lasting stimulatory effect on feeding and genetic over-expression of *Agrp* in the brain leads to obesity (Broberger and Hokfelt, 2001; Ollmann and Barsh, 1999; Rossi et al., 1998; Shutter et al., 1997). AgRP may promote feeding both by antagonizing the effect of α-MSH and by acting as an inverse agonist to suppress the constitutive activity of MC_3R and MC_4R in the absence of melanocortin input (Adan and Kas, 2003; Haskell-Luevano and Monck, 2001; Nijenhuis et al., 2001). However, knockout of the *Agrp* gene has little effect on body weight, food intake or adiposity (Qian et al., 2002). Furthermore, inactivation of both *Npy* and *Agrp* genes has no effect on body weight regulation (Qian et al., 2002). Therefore, the physiological relevance of NPY and AgRP in regulation of appetite and energy homeostasis remains to be established (Flier, 2006). One explanation for these disparate results is that regulatory pathways governing vital physiological functions such as feeding may be redundant, such that inactivation of one or even multiple genes, such as *Npy* and *Agrp*, during developmental process may promote compensatory mechanisms that mask the crucial functions conferred by these genes (Flier, 2006; Palmiter et al., 1998). Similar adaptive mechanisms may also exist in the adult, such that pharmacological intervention may have transient effects on feeding, but chronic treatment with the same drug may promote adaptive changes that diminish their effectiveness. Genetic inactivation of *Npy* gene expression in adult mice led to a 5-fold decline in *Npy* mRNA and protein from normal levels, but had little effect on feeding or body weight, perhaps because adaptive changes occurred during the slow depletion of NPY protein (Ste Marie et al., 2005). Experiments described below help to establish the existence of potent adaptive (compensatory) mechanisms.

Several groups devised strategies to ablate AgRP neurons to determine if the neurons are important for body weight regulation even if the peptides are dispensable (Bewick et al., 2005; Gropp et al., 2005; Luquet et al., 2005; Xu et al., 2005). Collectively the results indicate that AgRP neurons are important for maintaining feeding in adult mice, but that adaptive mechanisms can compensate for the loss of the neurons. Two of the ablation methods result in a slow progressive loss of AgRP neurons in adult mice due to targeted expression of a neurotoxic form of ataxin-3 (Bewick et al., 2005) or inactivation of a mitochondrial transcription factor *Tfam* gene (Xu et al., 2005). In both of these cases there is a small, but significant, reduction in body weight of adult mice. More rapid ablation of AgRP neurons in adult mice was achieved by expression of the human diphtheria toxin receptor selectively in AgRP neurons and the administration of diphtheria toxin (DT) to adult mice (Gropp et al.,