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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.,

2005; Luquet et al., 2005). In one study, the mice manifested severe anorexia and lost 20% of body weight within ~6 days and would die without intervention (Luquet et al., 2005). In a similar study, anorexia was evident and the mice became lean but starvation was not reported, possibly because the *Agrp-Cre* transgene was not expressed in all AgRP neurons so that enough AgRP neurons were spared to stave off starvation. DT treatment of mice on a *Npy*/*Agrp*-null background also leads to severe anorexia, indicating that loss of something other than these two neuropeptides is responsible (Phillips and Palmiter, 2008). Importantly, ablation of AgRP neurons in neonatal mice is tolerated as indicated by the fact that the ablated mice are only slightly smaller than normal as young adults. When examined as adults these mice have <15% of the normal number of NPY-expressing neurons in the arcuate nucleus, but when the same coronal sections are stained with AgRP antibodies the signal is absent suggesting that the remaining cells might express NPY only (Luquet et al., 2005, 2007). Moreover, most of the adult mice are resistant to a second injection of DT, and the orexigenic response triggered by ghrelin is gone throughout adulthood (Luquet et al., 2005, 2007). These results indicate that the AgRP neuron function is not important during the pre-weaning period, though this might be expected because they do not establish their mature axonal projections until the time of weaning (Bouret et al., 2004a). Moreover, ablation of AgRP neurons at a neonatal age must facilitate some compensatory mechanism that allows the adults to eat adequately without them—either by adaptations within the normal circuitry or by engaging alternative circuits (Luquet et al., 2005, 2007; Wu et al., 2009). A recent study indicated that progressive degeneration of AgRP neurons in adults triggers a neurogenesis mechanism in the hypothalamus leading to regeneration of AgRP neurons capable of responding to leptin (Pierce and Xu, 2010). It will be important to determine whether these regenerated AgRP neurons express all of the normal functions of AgRP neurons and whether they project to all of the normal targets. Despite the minimal effect of neonatal or gradual adult ablation of AgRP neurons on body weight, these mice manifest defects in their responses to ghrelin, food palatability (Luquet et al., 2007), and estrogen (Olofsson et al., 2009). We suggest that compensation for loss of AgRP neurons takes ~10 days; thus, if these neurons are ablated before they are functionally engaged (neonatal ablation) or slowly in the adult, then there is time for the adaptive mechanisms to maintain feeding. However, treating adult $Agrp^{DTR}$ mice with DT results in dysfunctional neurons within a couple of days and almost total loss of AgRP and NPY markers by 6 days (Wu et al., 2008b). It is likely that disruption of neural circuits occurs so fast that the mice die of starvation before adaptive mechanisms can be established.

2.1. AgRP neurons mediate anorexia independently of melanocortin signaling

Numerous studies indicate that AgRP neurons not only inhibit local POMC neurons by concerted antagonistic actions of GABA and NPY, but they also send collateral projections to many different brain areas that are coincident with POMC neuron projections (Broberger et al., 1998; Cone, 2005; Cowley et al., 1999; Haskell-Luevano et al., 1999; Horvath et al., 1997; Jacobowitz and O'Donohue, 1978; Watson et al., 1978). This arrangement makes sense if AgRP acts by antagonizing the effect of α-MSH on MC_3R and MC_4R. Consequently, the severe anorexia phenotype could be a consequence of disinhibition of the melanocortin signaling at one or more of the post-synaptic targets of AgRP neurons. Indeed, immunological analysis indicates that POMC expression is enhanced after AgRP neurons are ablated (Luquet et al., 2005, 2007) and electrophysiological recordings demonstrate that ablation of AgRP neurons reduces inhibitory input onto POMC neurons up to 6 fold (Wu et al., 2008a). However, contrary to expectations, behavioral data indicate that chronic blockade of the melanocortin pathway in $A^{y/a}$ genetic background (in which agouti protein is ectopically expressed in the brain thereby chronically blocking MC_4R signaling) failed to ameliorate the severe anorexia after ablation of AgRP neurons, suggesting that a melanocortin-independent mechanism underlies the starvation phenotype (Wu et al., 2008a). Enhanced release of other transmitters, such as, CART, GABA, glutamate, or other cleavage products of POMC may be co-released with α-MSH from different subsets of POMC neurons, and could potentially mediate the anorexia after loss of inhibition from AgRP neurons.

2.2. Post-synaptic neurons are differentially regulated by AgRP neurons

Conditional ablation of AgRP neurons upon DT treatment proved to be a useful tool to functionally profile post-synaptic targets that are most sensitive to loss of inhibitory inputs from AgRP neurons (Wu et al., 2008b). Because activated neurons often exhibit rapid accumulation of inducible transcription factors, such as Fos (Curran and Morgan, 1995), we employed a high-throughput in situ hybridization protocol to examine changes of these excitatory markers in brain areas with detectable AgRP-immunoreactive fibers (Wu et al., 2008b). The quantitative results indicated that post-synaptic targets of AgRP neurons can be functionally categorized into three groups: those with dramatic Fos activation that is attenuated in the $A^{y/a}$ background, including the arcuate, paraventricular nucleus, dorsal medial nucleus of the hypothalamus, medial preoptic nucleus, and lateral septum; those with no Fos activation (after normalized to pair-fed controls), including the raphe nucleus, ventral tegmental area, and bed nucleus of the stria terminalis; and those in which Fos activation persists in $A^{y/a}$ background, including the dentate gyrus, and nucleus of solitary tract (Wu et al., 2008b). Because blockade of melanocortin signaling by ectopic expression of agouti protein attenuates of Fos levels in most hypothalamic targets, those targets of AgRP neurons (e.g. the paraventricular and medial preoptic nuclei) likely receive balanced input from AgRP and POMC neurons.

The hippocampal dentate gyrus is not a direct target of AgRP neurons; however, the expression of Fos in this brain region is intriguing because it might allow homeostatic signaling to influence cognitive processes in the hippocampus. The orexigenic hormone ghrelin directly targets neurons of the hippocampal formation, where it promotes dendritic synapse formation and generation of long-term potentiation (Diano et al., 2006). Likewise, the endocannabinoid system plays a critical role in the regulation of food intake and energy expenditure through a unique retrograde mechanism to modulate synaptic transmission and plasticity (Matias and Di Marzo, 2007). One recent report suggests that mice specifically lacking cannabinoid 1 receptors on GABAergic neurons within the hippocampal CA1 and CA3 regions displayed partial resistance to diet-induced obesity, whereas chronic exposure to high-fat diet altered cannabinoid 1-mediated synaptic plasticity in the same areas (Massa et al., 2010). Thus, a chronic change in metabolic state, mediated in part by AgRP neuron circuitry may trigger adaptive changes and/or de novo neurogenesis in hippocampus which in turn contributes to the permanent alteration of appetitive behaviors (Dietrich and Horvath, 2009; Horvath, 2006). A large amount of evidence indicates an essential role of the nucleus tractus solitarius as a primary center for detection and transduction of gustatory and visceral sensory signals (Grill, 2006). Reciprocal interconnections between the nucleus tractus solitarius and hypothalamic cell groups including the POMC and AgRP neurons have been documented (Berthoud, 2002). The nucleus tractus solitarius is an extremely heterogeneous structure and it readily responds to vagal and hormonal inputs through a variety of signaling systems, such as glutamate, GABA, melanocortin, NPY, and glucagon-like peptides (Grill and Kaplan, 2002). It will be important to discern the identity of the Fos-positive cells in the nucleus tractus solitarius after AgRP neuron ablation and their corresponding ascending and descending fibers.

Some brain regions such as the bed nucleus of the stira terminalis and ventral tegmental nucleus, showed minimal or no Fos induction when AgRP neurons were ablated, although they were clearly innervated by AgRP neurons (Wu et al., 2008b). Their functional relevance to the hypothalamic AgRP circuitry remains uncertain. Several possible scenarios may underlie the lack of significant change of Fos induction. First, some neurons may display only a transient surge in activity that was missed by examining Fos at only one time point. Second, some targets of AgRP neurons may receive other inhibitory inputs that are strong enough to mask the loss of inhibitory projections from AgRP neurons. Third, the intracellular signaling substrates that are required to show Fos induction may be absent in some post-synaptic targets of AgRP neurons.

Immunohistochemistry revealed a robust gliosis response by both astrocytes and microglia that was associated with Fos induction in post-synaptic targets of AgRP neurons following DT-mediated ablation and it was often preceded by the degeneration of AgRP axons (Wu et al., 2008b). The gliosis might be related to degeneration of axonal fibers or to excitotoxicity due to hyperactivity of post-synaptic cells, or both. This observation raised the concern that gliosis and subsequent release of cytokines might be responsible for starvation in this model because some pro-inflammatory cytokines are known to inhibit feeding under certain conditions (Arruda et al., 2010; DeBoer et al., 2009; Jang et al., 2010; Laviano et al., 2008; Ramos et al., 2004). However, ablation of POMC neurons, which reside with AgRP neurons in the arcuate and project to virtually all of the same brain regions as AgRP neurons, leads to obesity (Gropp et al., 2005). Moreover, microglial activation persists in the hypothalamus even when feeding and body weight are completely rescued by chronic benzodiazepine treatment in AgRP neuron ablated mice (Wu et al., 2009). From this perspective, gliosis could elicit neurotrophic actions that attenuate hyperexcitability of post-synaptic neurons and thereby facilitate the establishment of a compensatory circuitry that restores appetite.

2.3. GABA signaling from AgRP neurons within the PBN prevents anorexia

Pharmacological studies suggest that both central $GABA_A$ and $GABA_B$ receptor signaling exert prominent influences on feeding in various brain regions (Berridge, 2009; Cooper, 2005; Duke et al., 2006; Martire et al., 2010; Pecina and Berridge, 1996; Stratford and Kelley, 1997). A class of benzodiazepine agonists, which acts through a specific binding site on $GABA_A$ receptors, induces hyperphagia by enhancing food and taste palatability in every mammalian species studied (Cooper, 2005). For example, in rodents the benzodiazepine agonist chlordiazepoxide increases the consumption of palatable solutions (e.g. saccharin) over water and promotes the facial 'liking' reactions elicited by intraoral infusion of a palatable solution (Berridge and Treit, 1986; Cooper, 1987; Cooper and McClelland, 1980). Although a hypothalamic site of action was initially proposed for benzodiazepine-induced appetite (Anderson-Baker et al., 1979; Kelly and Grossman, 1979), more compelling evidence suggested that the PBN, a brainstem structure, near the 4th ventricle, is more relevant, because direct administration of midazolam (another benzodiazepine agonist) into the PBN elicited strong hyperphagic response and enhanced taste palatability (Higgs and Cooper, 1996; Soderpalm and Berridge, 2000b). Notably, benzodiazepines do not initiate similar effects on feeding and taste reactivity when delivered to some nearby areas including the nucleus tractus solitarius, pedunculo-pontine tegmental nucleus, or nucleus accumbens (Soderpalm and Berridge, 2000a; b). Peripheral or intracranial administration of the $GABA_B$ agonist baclofen increases short-term (within 2 hours), but not daily, food consumption in satiated rats, and has no effect on the body weight (Ebenezer and Patel, 2004; Patel and Ebenezer, 2008). Conversely, intracranial administration of $GABA_B$ antagonist CGP-35348 reduces food consumption in fasted rats in a dose-dependent manner (Patel and Ebenezer, 2004). Results derived from site-directed microinjection of a $GABA_B$ agonist baclofen in the nucleus accumbens or median raphe nucleus suggested that both areas were possible sites mediating the stimulatory effect of $GABA_B$ signaling on food intake (Stratford and Kelley, 1997; Ward et al., 2000; Wirtshafter et al., 1993).

GABA release from AgRP neurons has a direct inhibitory effect on POMC cells and probably on most post-synaptic MC4R-bearing cells (Cowley et al., 2001; Horvath et al., 1997; Pu et al., 1999; Wu et al., 2008a). GABA co-localizes with NPY–immuno-positive axon terminals that innervate local POMC neurons in the arcuate as well as both parvocellular and magnocellular divisions of the paraventricular nucleus that receive dense projection from AgRP and POMC neurons (Cowley et al., 2001; Horvath et al., 1997; Pu et al., 1999). Electrophysiological recordings indicate that leptin inhibits release of GABA from NPY terminals that synapse onto POMC neurons (Cowley et al., 2001). Based on these observations, we suspected that GABA output from AgRP neurons may be important to maintain a dynamic balance with excitatory signaling in certain post-synaptic target areas.

We recently demonstrated that GABA release from AgRP neurons onto neurons in the lateral PBN of the hindbrain is essential for maintenance of appetite and body weight (Wu et al., 2009). The severe anorectic response resulting from ablation of AgRP neurons can be prevented by chronic infusion (via 14-day Alzet minipump) of a benzodiazepine partial agonist, bretazenil, directly into the PBN or into the 4th ventricle (Wu et al., 2009). Bretazenil infusion completely prevents Fos induction in the PBN area when AgRP neurons are ablated (Wu et al., 2009). Injecting DT bilaterally into the PBN, recapitulates the severe anorexia observed when DT is administered peripherally, indicating that AgRP neurons express DT receptor on the axonal terminals in the PBN (Wu et al., 2009). We have also shown that inhibition of GABA signaling in the PBN of normal mice with the $GABA_A$ antagonist, bicuculline, inhibits feeding in a dose-dependent manner (Wu et al., 2009). To help establish AgRP neurons as the source of the GABA signaling in the PBN, we inactivated GABA biosynthesis in the arcuate by delivering a virus that would inactivate the biosynthetic enzymes. When the virus was delivered directly into the arcuate, the mice became anorexic and lost body weight, but the effect was not as severe as DT ablation, presumably because it is difficult to transduce all AgRP neurons by viral injection (Wu et al., 2009). Genetic inactivation of the vesicular GABA transporter gene in AgRP neurons produces mice with a lean phenotype that are resistant to diet-induced obesity, yet have normal food intake (Tong et al., 2008). We suggest that the inactivation of the GABA vesicular transporter is less effective than the viral approach because it occurs during development and hence promotes compensatory mechanisms. The combined results suggest that the sudden loss of GABA signaling by AgRP neurons in adult mice leads to hyperactivity of a subset of lateral PBN neurons (manifest as Fos activity) and that the synaptic output of those hyperactive PBN neurons provides a potent brake on some essential feeding circuit. Ameliorating the hyperactivity of PBN neurons by chronic administration of bretazenil, a GABAA receptor partial agonist prevents the severe anorexia. The hyperactivity of PBN neurons is presumably due to the unopposed excitatory drive onto them; the neurotransmitter(s) involved in that excitation and their source is the subject of current investigations.

Another paradigm where Fos activation occurs in the lateral PBN is conditioned taste aversion. In this paradigm, mice are exposed to a novel taste followed by LiCl injection into the peritoneum, a procedure that mimics gastrointestinal malaise that is typically associated with food poisoning (Grill, 1985; Spector et al., 1988). As a consequence, mice learn with only one trial to avoid that taste in future trials. This is clearly an important adaptive response to prevent consumption of foul or toxic food. Ablation of AgRP neurons not only inhibits the initiation of feeding, but also inhibits consumption of palatable food (condensed milk or sugar water) delivered directly into the mouth via an intraoral

fistula (Wu et al., 2008a). Thus, AgRP-ablated mice will not swallow very much when milk or sucrose solution pumped directly into their mouth even though they are starving, whereas food consumption actually increases in fasted mice. This consummatory behavior is thought to be regulated by a taste-sensing circuitry within brainstem structures including the nucleus tractus solitarius and PBN (Grill, 2006; Grill and Kaplan, 2002). The deficits in consummatory response to palatable food in mice with ablated AgRP neurons can be restored by the infusion of bretazenil (Wu et al., 2009). These results suggest that there is significant crosstalk between the hypothalamic energy balance-sensing circuitry and the hindbrain visceral and taste-sensing network including the nucleus tractus solitarius and PBN, which might orchestrate feeding behavior.

2.4. Adaptation to loss of AgRP neurons

Emerging evidence suggests that neural networks involved in control of feeding can adapt to changes of hormone levels in periphery or metabolic state (Bouret et al., 2004b, 2008; Horvath et al., 2004; Horvath and Gao, 2005; Kokoeva et al., 2005; Pinto et al., 2004; Simerly, 2005). For example, $Lep^{ob/ob}$ mice display significant differences from wild-type mice with respect to excitatory and inhibitory inputs onto POMC and AgRP neurons, which can be rapidly normalized upon leptin treatment (Pinto et al., 2004). Leptin treatment in $Lep^{ob/ob}$ neonates, but not in adults, restores normal projection patterns to the arcuate (Bouret et al., 2004b). These data indicate that leptin mediates formation of proper connections among hypothalamic components in feeding circuits. In line with this line of thinking, feeding persists in AgRP neuron-ablated mice after the bretazenil-eluting mini pumps are depleted or removed, indicating that GABA signaling may promote long-lasting synaptic rearrangements at post-synaptic PBN neurons that counteract excitability induced by loss of inhibitory projections from AgRP neurons (Wu et al., 2009). Indeed, enhanced expression of glutamate decarboxylase, GAD67, the rate-limiting enzyme for GABA biosynthesis, increases dramatically in the PBN after ablation of AgRP neuron and may reflect one such compensatory change (Wu et al., 2009). We suggest that benzodiazepine treatment dampens PBN neuronal activity and thereby allows adaptive responses. Future studies are aimed at identifying changes in the synaptic inputs to PBN neurons and their outputs. It is also likely that there are adaptive changes in synaptic efficacy (synaptic plasticity) within the relevant PBN neurons after AgRP neuron-ablation.

Benzodiazepines have been shown to modulate synaptic plasticity in other brain areas. For example, chronic treatment of benzodiazepine restores long-term depression in the visual cortex of *Gad2* (the gene encoding of another glutamate decarboxylase isoform, GAD65) knockout mice, whereas benzodiazepine inverse agonists augment long-term potentiation in hippocampal slices (Choi et al., 2002; Yasui et al., 1993). Taste-responsive neurons in the PBN receive predominant GABA innervation from forebrain areas, including gustatory cortex, the bed nucleus of the stria terminalis, central nucleus of the amygdale and lateral hypothalamus, which are all involved in the modulation of various taste-evoked responses by the PBN, like lithium-associated conditioned taste aversion (Jia et al., 2005; Kobashi and Bradley, 1998; Lundy and Norgren, 2004; Moga et al., 1990a,b). These results suggest that the PBN neurons utilize forebrain GABA input to discriminate and organize hedonic signals with sensory stimuli from the tongue and gut, thereby allowing proper interpretation of food palatability and satiety signals with signals from the arcuate related to energy balance.

2.5. AgRP neurons may impact brain hedonic systems in control of feeding

The central homeostatic system featured by POMC and AgRP pathways reciprocally interact, at multiple levels, with hedonic mechanisms including sensory inputs from taste and smell as well as brain reward systems, such as dopamine, endocannabinoid, and opioid pathways (Berthoud, 2002; Cooper, 2004; Kelley et al., 2005; Palmiter, 2008; Saper et al., 2002). Enhanced feeding induced by AgRP or *NPY* administration can be blocked by opioid receptor antagonist naloxone (Hagan et al., 2001). Transgenic mice that lack the ability to produce dopamine normally become severely hypophagic; they fail to respond to glucoprivation, peptide YY, or leptin deficiency, reminiscent to phenotypes found in AgRP neuron-ablated mice (Hnasko et al., 2004; Luquet et al., 2007; Szczypka et al., 2000; Zhou and Palmiter, 1995). Considering that dopamine signaling provides a permissive signal for feeding that apparently acts downstream of AgRP neurons signaling (because dopamine-deficient mice fail to respond to peptide YY), it will be important to determine where the dopamine and AgRP signaling pathways intersect to control feeding. Likewise, elucidating the physiological interactions between AgRP circuitry and serotonin, opioid, or vagal afferent signals will advance our understanding of how the hedonic and homeostatic circuits control feeding behaviors.

3. A new perspective

The AgRP neuron-ablation experiments have had several substantial consequences on our thinking about control of feeding behavior. First, we now argue that GABA signaling in the AgRP neurons plays a critical role in maintaining feeding behavior of adult mice reared on a normal chow diet. Previously, most of the attention had been directed towards the co-releasing neuropeptides NPY and AgRP. Second, we have learned that compensation for loss of AgRP neurons occur if the neurons are ablated in neonatal mice, as well as in adult mice if the ablation occurs slowly, and in adult mice where ablation is rapid but the anorexia is suppressed with bretazenil. A parsimonious assumption is that the same adaptive mechanism(s) are engaged in all three conditions, but until the mechanisms are characterized, it is not possible to answer this question. If mice can adapt to the loss of AgRP neurons, then it follows that they can adapt to the loss of any transmitter made by those neurons, including NPY, AgRP, or GABA. Third, adaptation to loss of AgRP neurons allows mice to eat adequately, but the adaptive mechanisms alter their responses to hormones like ghrelin, diet palatability and probably many other environmental influences. Fourth, the identification of the PBN as a critical target of GABA signaling by AgRP neurons provides a bridge between the homeostatic system in the arcuate and the gustatory signals from the mouth and visceral inputs from gut. Identifying the relevant neurons within the PBN that control feeding, their synaptic inputs as well as the brain regions they control remain as challenging problems. A better understanding of the molecular mechanism(s) underlying the adaptive mechanisms and the unique features of $GABA_A$ signaling in the PBN should facilitate a better understanding of the neural regulation of feeding behavior, and perhaps aid in devising new treatments for eating disorders.

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