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REVIEW

Expanding neurotransmitters in the hypothalamic neurocircuitry for energy balance regulation

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ABSTRACT

The current epidemic of obesity and its associated metabolic syndromes impose unprecedented challenges to our society. Despite intensive research on obesity pathogenesis, an effective therapeutic strategy to treat and cure obesity is still lacking. Exciting studies in last decades have established the importance of the leptin neural pathway in the hypothalamus in the regulation of body weight homeostasis. Important hypothalamic neuropeptides have been identified as critical neurotransmitters from leptin-sensitive neurons to mediate leptin action. Recent research advance has significantly expanded the list of neurotransmitters involved in body weight-regulating neural pathways, including fast-acting neurotransmitters, gamma-aminobutyric acid (GABA) and glutamate. Given the limited knowledge on the leptin neural pathway for body weight homeostasis, understanding the function of neurotransmitters released from key neurons for energy balance regulation is essential for delineating leptin neural pathway and eventually for designing effective therapeutic drugs against the obesity epidemic.

KEYWORDS obesity, leptin, neurotransmitter, hypothalamus

INTRODUCTION

Obesity is one of the greatest and most challenging problems in medicine and is a leading risk factor for metabolic syndromes characteristic of type II diabetes mellitus, hypertension, stroke, and coronary heart disease (Patel et al., 2008). In humans, the body weight status is measured by body mass index (BMI), which is defined as body weight in kilograms divided by height in meters. Subjects with a BMI more than 30 are considered to be obese. Body weight homeostasis is achieved by complex interactions between the brain and peripheral tissues, and is maintained by balanced energy intake (food intake) and energy expenditure (Flier, 2004). Obesity develops when energy intake exceeds energy expenditure. The obesity epidemic has been occurring at an alarming rate and by year 2030, it is projected that nearly half of Americans will be obese (Wang et al., 2011). The economic cost imposed by obesity has become a substantial burden to the society, which signifies an urgent need for efficient therapeutics to prevent and treat obesity (Swinburn et al., 2011; Wang et al., 2011). To achieve this, it is critical to understand the function of key neurons and neurotransmitters within body weight-regulating neural pathways. Extensive research in the last decades has identified important brain neurons and neurotransmitters, especially those in the hypothalamus, for body weight regulation. This review briefs recent advance in brain mechanisms on body weight homeostasis, with a special focus on the function of neurotransmitters within the leptin neural pathway in the hypothalamus (see a simplified diagram in Fig. 1).

THE HYPOTHALAMUS AND OBESITY

The hypothalamus receives and integrates a variety of inputs from both peripheral tissues and other brain regions, and then in turn sends output commanding signals to generate coordinated responses for the maintenance of body home-

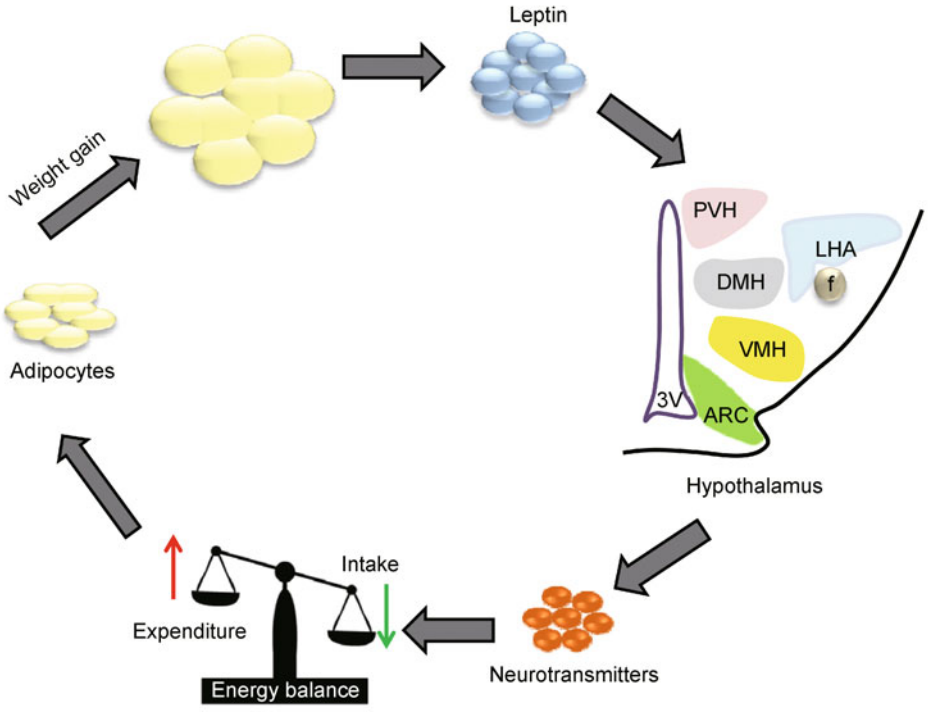

Figure 1. Hypothalamic leptin pathways and body weight regulation. Increased fat mass due to increased body weight leads to increased levels of leptin, which will activate the leptin neural pathway in the brain, especially in the hypothalamus. The hypothalamic neurons, including those in Arc, VMH, DMH, PVH and LH will alter release of neurotransmitters, which will then act on downstream neurons to initiate coordinated responses to reduce feeding and increase energy expenditure, and to reduce fat mass, thus achieving body weight homeostasis. Arc: the arcuate nucleus; VMH, ventromedial hypothalamus; DMH: dorsomedial hypothalamus; PVH, paraventricular hypothalamus; LH: lateral hypothalamus; f: fornix; 3V: the third ventricle.

ostasis (Morrison and Berthoud, 2007; Marino et al., 2011). It has long been recognized as a critical site for body weight regulation. Earlier lesion studies have established the ventromedial hypothalamus (VMH) as a satiety center and lateral hypothalamus (LH) as a hunger center in the brain (Brobeck, 1946; Teitelbaum and Epstein, 1962), In addition, lesion of the arcuate nucleus (Arc) or the paraventricular hypothalamus (PVH) also produces hyperphagia and obesity (Lorden and Caudle, 1986; Touzani and Velley, 1992). Furthermore, a knife cut between the hypothalamus and the hindbrain also causes obesity, suggesting an important role for the neuronal projections between these two brain structures in body weight regulation (Kirchgessner and Sclafani, 1988). Collectively, these studies demonstrate that body weight homeostasis is regulated by a distributed neural network in the hypothalamus.

HYPOTHALAMIC LEPTIN NEURAL PATHWAY AND ENERGY BALANCE

The discovery of leptin by Douglas Coleman and Jeffrey Friedman, which was credited with the Lasker Award in 2010, has opened a new era toward understanding the mechanisms underlying body weight homeostasis (Coleman and Hummel, 1969; Coleman, 1973; Zhang et al., 1994; Flier and Maratos-Flier, 2010). Leptin, a 16-kDa protein, is secreted from adipocytes in proportion to the mass of adipose tissues. The importance of leptin is manifested by massive obesity and severe diabetes as well as an array of other endocrine and immunological diseases in subjects with inactivation of leptin (Montague et al., 1997). Consistently, mice with deficiency in leptin (*ob/ob*) exhibit severe obesity, hyperphagia, reduced energy expenditure, short statue, lower bone density, sterile and diabetes (Bates and Myers, 2003), demonstrating that mice and humans share a common leptin pathway. The function of leptin is mainly mediated by the B isoform of leptin receptors (denoted as LepRb) since a similar phenotype to leptin deficiency was observed in mice (*db/db*) and humans with LepRb inactivation (Chen et al., 1996; Lee et al., 1996; Clément et al., 1998). Despite strong evidence showing LepRb expression in other sites, the brain is the major site that mediates the function of leptin since brain-specific deletion of LepRb largely recapitulates phenotypes with whole body LepRb deletion (Cohen et al., 2001). Reciprocally, abnormalities of the *db/db* mouse are markedly improved by transgenic replacement of central LepRbs (de Luca et al., 2005). Consistent with this, liver-specific deletion

of LepRb produces little effects (Cohen et al., 2001).

Within the brain, the hypothalamus exhibits the most abundant expression of LepRb (Schwartz et al., 1996). Outside the hypothalamus, LepRb is only expressed in scattered neurons in the cortex, hippocampus, periaqueductal grey, dorsal raphe, the hindbrain and other sites (Scott et al., 2009; Patterson et al., 2011). Within the hypothalamus, the Arc shows the highest density of LepRb expression while the ventral premammillary nucleus (PMv), VMH and the dorsal medial hypothalamus (DMH) also show prominent expression (Scott et al., 2009; Patterson et al., 2011). This expression pattern is in line with anatomical location of these nuclei relative to the median eminence (ME) and with their accessibility to leptin (Faouzi et al., 2007). Collectively, the hypothalamus is the major site in the brain that mediates leptin action on energy balance regulation.

MULTIFACETED ROLE OF LEPTIN

Both leptin deficient *ob/ob* and LepRb deficient *db/db* mice show severe diabetes and other abnormalities including reduced growth, bone defects and altered reproduction, in addition to obesity (Takeda et al., 2003; Bjørbaek and Kahn, 2004; Hill et al., 2008; Myers et al., 2009), suggesting a multifaceted role of leptin. For example, accumulating data suggest that leptin action in the brain directly regulates glucose homeostasis. Specific re-expression of *LepRb* in the Arc dramatically rescues the diabetic phenotype of *db/db* mice, and this effect is not associated with body weight changes (Coppari et al., 2005). Specifically, over-expression of LepRb selectively in proopiomelancortin (POMC) neurons, which are one group of well-characterized LepRb-expressing neurons in the Arc, largely restores euglycemia in *db/db* mice (Huo et al., 2009). The most convincing evidence to support direct leptin action on glucose homeostasis is from studies on type 1 diabetes. In type 1 diabetic models, due to damage of pancreatic beta-cells and lack of insulin, the glucose concentration is uncontrolled and dramatically increased. Strikingly, both peripheral and central administration of leptin effectively lowers glucose to a normal range, which is independent of its effects on body weight (Yu et al., 2008; Fujikawa et al., 2010; Wang et al., 2010; German et al., 2011). Interestingly, unlike insulin, which imposes the risks of hypoglycemia and lipid imbalance, the leptin action improves lipid profile without hypoglycemia risk (Fujikawa et al., 2010; Wang et al., 2010; Kruger et al., 2011). Consistent with a role of central leptin, *LepRb* expression in liver is not required for leptin action on euglycemia restoration in type 1 diabetes (Denroche et al., 2011).

The multifaceted role of leptin may be due to the fact the leptin neural pathway is shared by other important nutritional cues including glucose, lipid and insulin (Blouet and Schwartz, 2010). The Arc, which shows the most abundant expression of LepRb, is a critical site for glucose and lipid sensing, both of which are mediated through projections from the hypothalamus to liver (Lam et al., 2005a, 2005b; Pocai et al., 2005; Marino et al., 2011). Specifically, POMC neurons, agouti-gene related protein (AgRP) neurons, another well established group of LepRb-expressing neurons in the Arc, and a subset of neurons in the VMH, are capable of directly sensing glucose (Song and Routh, 2005; Parton et al., 2007; Cheng et al., 2008). Although the mechanism underlying glucose-induced inhibition is not clear, glucose induced excitation involves inhibition of K_{ATP} channel elicited by increased ATP levels, which can be modulated by uncoupling protein (UCP) activity (Parton et al., 2007; Kong et al., 2010). Strikingly, insulin action in the hypothalamus shows a dominant role on glucose homeostasis over that in the liver (Buettner et al., 2005; Okamoto et al., 2005). Consistently, insulin receptor expression in the hypothalamus is important for both body weight and glucose homeostasis (Obici et al., 2002a, 2002b). Given the importance for both insulin and leptin action in the hypothalamus on glucose homeostasis, one might expect that they act on the same neurocircuit. Indeed, insulin action on AgRP neurons is required for normal glucose homeostasis in the liver (Könner et al., 2007), and insulin and leptin signalings converge on the phosphoinositide-3 (PI3) kinase pathway in POMC and AgRP neurons (Xu et al., 2005). However, given the mild role of these neurons in glucose homeostasis, it might be possible that other key, yet to be identified neurons mediate the central action of insulin and leptin on glucose homeostasis. Especially, for POMC neurons, emerging data support a notion that insulin and leptin actually activate distinct subgroups of POMC neurons (Williams et al., 2010), which rules out the possibility of POMC neurons as candidate for mediating both leptin and insulin on glucose homeostasis. This notion is supported by normal glucose homeostasis observed in mice with specific deletion of *LepRb* in POMC neurons (Balthasar et al., 2004). The existence of key, yet to be identified neurons that mediate insulin and leptin action is supported by studies on Rip-Cre neurons, which are a distinct group of neurons from POMC and AgRP neurons (Choudhury et al., 2005; Song et al., 2010). Specific deletion of either insulin or leptin signaling in these neurons results in severe obesity and defects in glucose homeostasis (Kubota et al., 2004; Lin et al., 2004; Choudhury et al., 2005; Covey et al., 2006).

In addition, the leptin neural pathway mediates the action of ghrelin, serotonin, acetylcholine and others (Chen et al., 2004; Xu et al., 2010; Mineur et al., 2011). Thus, the leptin neural pathway integrates nutritional status from peripheral as well as inputs from other brain sites, and initiates a coordinated response to nutritional changes and other brain functions, to achieve energy homeostasis. How do these neurons initiate the coordinated response? For neurons, all received information has to be transmitted to the target neurons through changes of neurotransmitter release (Fig. 1). Thus, revealing the function of neurotransmitters released

from key body weight-regulating neurons is essential to delineation of neural pathways for energy balance regulation.

NEUROPEPTIDE NEUROTRANSMITTERS IN THE Arc

Exciting research advances in the last decades have revealed the important role for the melanocortin system in mediating leptin action on energy homeostasis. The melanocortin system includes POMC and AgRP neurons, both of which are located in the Arc, and the melanocortin receptors (MC4Rs and to a lesser degree MC3Rs), which are expressed not only in the PVH, but also in other brain sites including the amygdale (Elmquist et al., 2005). *Mc4r* is a well established anti-obesity gene and its dysfunction in both mice and humans causes hyperphagia, reduced energy expenditure and severe obesity (Huszar et al., 1997; Yeo et al., 1998). Despite recent evidence that a small subset of AgRP and POMC neurons share the same progenitor during development (Padilla et al., 2010), POMC and AgRP neurons are largely distinct from each other, at least in the adult stage. Upon activation, POMC neurons release α-melanocyte stimulating hormone (α-MSH), a peptide derived from POMC and an agonist for MC4Rs, and AgRP neurons release both AgRP, an endogenous antagonist for MC4Rs, and neuropeptide Y (NPY), a potent orexigenic peptide (Elmquist et al., 2005). Intriguingly, leptin activates POMC neurons while inhibiting AgRP neurons through the same LepRb (Morton, 2007). In addition, recent data suggest that leptin-mediated activation of POMC neurons involves not only direction activation, but also, in a greater degree, reduction of GABAergic input to POMC neurons by inhibiting a group of yet to be identified GABAergic neurons (Vong et al., 2011). Thus, increased leptin levels can effectively achieve food intake inhibition and boost energy expenditure through activation of MC4Rs by increasing alpha-MSH release and reducing AgRP release, and additionally through reducing NPY secretion.

The proposed role of the melanocortin system in mediating leptin action is supported by numerous anatomical, physiological, pharmacological and genetic studies. For example, POMC neurons (α-MSH) and AgRP neurons (AgRP and NPY) send strong projections to common targets where MC4Rs are located, such as the PVH (Mezey et al., 1985; Broberger et al., 1998). Compelling pharmacological data demonstrate that AgRP and NPY, when administered in the brain, produce powerful orexigenic effects, while α-MSH and its mimetic MTII produce anorexigenic effects (Kalra and Kalra, 2010). Electrophysiological data demonstrate that leptin activates POMC neurons while inhibiting AgRP neurons (Cowley et al., 2001), which is consistent with the data that leptin induces c-fos expression (an indicator for neuronal activation) in POMC neurons, but not in AgRP neurons, while inducing SOCS3 expression (an indicator for leptin receptor activation) in both groups of neurons (Elias et al., 1999). In further corroboration with this, fasting induces robust up-regulation of AgRP and NPY, and down-regulation of POMC, which coincides with decreased levels of leptin (Ahima et al., 1996; Breen et al., 2005; Takahashi and Cone, 2005), suggesting that the underlying mechanism for fasting-induced hyperphagia is inhibition of POMC neurons and "dis-inhibition" of AgRP neurons as a result of reduced leptin levels. Genetically, direct leptin action on POMC and AgRP neurons has been revealed by the obesity phenotypes caused by specific deletion of LepR in POMC and AgRP neurons (Balthasar et al., 2004; van de Wall et al., 2008). An increased α-MSH release from POMC neurons owing to changes in POMC prohormone post-translational modification reduces food intake and body weight (Plum et al., 2009), demonstrating a critical role for α-MSH as an anorexigenic neurotransmitter. Furthermore, MC4R inhibition as a result of genetic knockout of POMC or over-expression of AgRP (agouti mice) leads to severe obesity (Ollmann et al., 1997), demonstrating a powerful role for the melanocrotin system in body weight regulation.

Despite the above-mentioned strong evidence supporting the melanocortin model, a few recent studies have generated inconsistent data. If normal MC4R function is required for body weight homeostasis, then deletion of AgRP would cause defects. Given the potent orexigenic effect of NPY, one might expect that reduced NPY action would cause reduced feeding. However, surprisingly, mice with knockout of either AgRP or NPY or both produce little effects on feeding or body weight (Qian et al., 2002). Even more surprisingly, ablation of AgRP neurons in the neonatal stage produces little effects on feeding or body weight (Luquet et al., 2005). These results are in contrast with severe obesity caused by POMC deletion (Yaswen et al., 1999). It has been speculated that the brain has evolved multiple orexigenic pathways to combat famine during the evolution, and if one has defects, other pathways will compensate (Berthoud and Morrison, 2008). This speculation is consistent with the data that ablation of AgRP neurons in neonates produces little effects while that in adults produces strong starvation phenotype (Luquet et al., 2005). However, the starvation effect produced by AgRP neuron ablation is independent of the melanocortin system (Wu et al., 2008). Thus, it appears that the function of AgRP neurons is sufficient, but not required for body weight regulation, at least in neonates. It would be interesting to know the nature of the compensatory pathways that kick in for loss of AgRP neurons. Studies using inducible ablation of AgRP and NPY release from AgRP neurons in the adult stage are required to examine their *in vivo* function within the melanocortin system.

In addition to α-MSH, POMC neurons can also release other neuropeptides, including β-endorphin and adrenocorticotropin (ACTH), both of which are encoded by the same POMC gene, and cocaine- and amphetamine-regulated transcript peptide (CART). Earlier data suggest that β-

endorphin may stimulate feeding (Kalra et al., 1999), which appears to be contradictory to the anti-obesity function of POMC neurons. In contrast, a new study suggests that β-endorphin released from POMC neurons inhibits AgRP neurons (Yang et al., 2011). Interestingly, ACTH has been reported to inhibit feeding through MC4Rs in the PVH (Schulz et al., 2010). CART, in addition to its expression in POMC neurons, shows wide-spread expression in the brain including the hypothalamus (Hunter et al., 2004). Within the hypothalamus, CART is highly colocalized with LepRb and its expression is up-regulated by leptin (Kristensen et al., 1998), which is consistent with the role of CART in reducing feeding and the obesity phenotype due to its deletion (Hunter et al., 2004; Wierup et al., 2005). Despite the well-recognized anti-obesity action of CART, its post-synaptic action is largely unknown. A recent study indicates that CART may exert its action through G-protein coupled receptors (Lin et al., 2011). Thus, POMC neurons release multiple neuropeptides to mediate its anti-obesity effects.

Galanin-like peptide (GALP) and kisspeptin represent two additional important peptides released from the Arc. GALP is expressed in a small subset of Arc neurons (Ohtaki et al., 1999), and both leptin and insulin increase its expression levels (Juréus et al., 2000; Fraley et al., 2004). However, mice with GALP deletion exhibit normal body weight on chow and less body weight on high-fat diet (Dungan Lemko et al., 2008), suggesting a complicated role of GALP in body weight regulation. This may be related to massive projections from GALP neurons to other brain sites including orexin neurons in the LH, AgRP and POMC neurons in the Arc (Shiba et al., 2010). Kisspeptin is mainly expressed in the Arc and the anteroventral periventricular nucleus of the hypothalamus, and only a small subset of Kisspeptin neurons co-express LepRb (Cravo et al., 2011). Through action on its receptor GRP54, this peptide is mainly involved in reproduction, but not in body weight regulation (d'Anglemont de Tassigny et al., 2007). However, a recent study suggests that Kisspeptin might mediate only a limited role of leptin action on reproduction (Louis et al., 2011).

NEUROPEPTIDES IN OTHER HYPOTHALAMIC SITES

In addition to the Arc, other hypothalamic sites also release neuropeptides, which are directly or indirectly involved in the leptin neural pathway. Consistent with satiety center of VMH, deletion of LepRb in steroidogenic factor 1 (SF1)-expressing neurons causes obesity associated with hyperphagia (Dhillon et al., 2006). In line with this, expression levels of brain-derived neurothophic factor (BDNF) respond to nutritional status and deletion of VMH BDNF leads to obesity and hyperphagia (Xu et al., 2003; Unger et al., 2007), suggesting that BDNF mediates the action of VMH neurons in the regulation of energy balance. However, a normal body weight phenotype was observed in mice with deletion of BDNF in SF1 neurons (Tong et al., 2007), suggesting that BDNF expression in SF1 neurons is not required for normal body weight regulation, and that BDNF in non-SF1 neurons within the VMH may be important for body weight regulation. Apart from BDNF, recent results indicate that pituitary adenylate cyclase activating polypeptide (PACAP) is abundantly expressed in the VMH and its expression levels respond to nutritional status, suggesting a potential role for PACAP in body weight regulation (Hawke et al., 2009).

The neuropeptides orexins and melanin-concentrating hormones (MCH) show selective expression in the LH. Expression of both peptides increases in response to fasting and mice with deletion of each peptide exhibit hypophagia and reduced body weight (Sakurai et al., 1998; Shimada et al., 1998), which is consistent with the role of LH as a hunger center. Importantly, pharmacological administration of either orexins or MCH increases feeding and blockage of receptors of these peptides decreases feeding (Qu et al., 1996; Sakurai et al., 1998), demonstrating that both neuropeptides act as neurotransmitters mediating the role of LH neurons in body weight regulation. The underlying mechanism for orexin function in feeding involves increasing arousal and for MCH involves a projection to the nucleus accumbens, a brain region involved in reward regulation (Chemelli et al., 1999; Pissios et al., 2008). Notably, although LepRb is also expressed in the LH, neither orexin nor MCH neurons express LepRb (Leinninger et al., 2009). However, existing evidence supports that these neurons may directly receive input from LepRb-expressing neurons in the Arc or LH itself (Leinninger, 2011). Thus, orexins and MCH indirectly mediate leptin action through complicated neural networks.

As discussed before, the PVH receives intense projections from the Arc, where LepRbs are most abundantly expressed, suggesting that PVH neurons are downstream mediators for leptin action. In addition, despite relative lack of LepRb expression in the PVH, a recent study based on electrophysiology suggests that PVH neurons may also directly mediate leptin action (Ghamari-Langroudi et al., 2011). Within the PVH, distinct subsets of neurons express oxytocin, thyrotropin releasing hormone (TRH) or corticotrophin releasing hormone (CRH) among others. Substantial data have been accumulated that support a role for oxytocin in regulating body weight. Administration of oxytocin produces hypophagia while administration of oxytocin receptor antagonists results in hyperphagia (Zhang et al., 2011a). Notably, evidence supports a model that oxytocin release in the nucleus of solitary tract (NTS) through projects from the PVH modulates the activity of local neurons, which fine gauges the activity of these neurons in response to food intake-regulating hormones (Blevins et al., 2004). In addition, defects in oxytocin release are possibly responsible for the obesity owing to the haploinsufficiency of single-minded 1, a transcription factor required for PVH development (Kublaoui

et al., 2008). Of note, a recent study convincingly demonstrated that the regulated oxytocin release by snaptotagmin 4 modulates feeding and defects in this regulation mediate diet-induced obesity (Tong, 2011; Zhang et al., 2011a). For TRH and CRH, both pharmacological and physiological studies suggest that they may play an important role in energy balance regulation (Kalra and Kalra, 2010). However, mice with knockout of either peptide exhibit normal or reduced body weight (Venihaki and Majzoub, 1999; Yamada et al., 2003). These inconsistent data may be due to profound developmental roles of these neuropeptides, which may mask their role in physiological regulation of body weight in the adult stage.

The DMH expresses abundant LepRb and shows robust p-STAT3 expression in response to leptin action (Scott et al., 2009), suggesting an important function for DMH neurons in mediating leptin action. Despite this, the specific role of LepRb in the DMH in body weight regulation remains to be demonstrated. Nonetheless, compelling evidence supports a role for NPY release from DMH neurons in mediating energy balance (Chao et al., 2011).

ROLE OF FAST-ACTING NEUROTRANSMITTERS IN MEDIATING LEPTIN ACTION

Fast-acting neurotransmitters, GABA and glutamate, account for most of the synaptic activity and intercellular signaling in the brain, including the hypothalamus. Importantly, blocking ionotropic GABA and glutamate receptors with selective antagonists generally eliminated most or all fast, miniature, and evoked synaptic activity in hypothalamic slices or cultures (van den Pol, 2003). Given their essential role in neurotransmission, it is surprising that the function of GABA and glutamate in mediating energy balance is largely neglected until recently. The underlying reason for the neglect is not because these neurotransmitters are thought not to be important for energy balance regulation, but rather because it is challenging to examine their *in vivo* function. The difficulties lie in the universal existence of GABAergic and glutamatergic neurotransmission and global defects in their neurotransmission are often incompatible with life, eliminating the opportunity to study their roles in physiology. Because essentially all neurons have receptors for GABA and glutamate, site-specific injections of agonists or antagonists invoke responses in all neurons exposed, generating results of unclear physiologic meaning.

As discussed above, extensive studies have been focused on selected groups of LepRb neurons with known transcription markers, including POMC, AgRP, NPY and SF1 (van den Pol, 2003; Elmquist et al., 2005; Dhillon et al., 2006; van de Wall et al., 2008; Morris and Rui, 2009). However, evolving studies suggest that these neurons only account for a small fraction of total LepR neurons and mediate limited leptin action on body weight regulation (Dhillon et al., 2006; van de Wall et al., 2008; Vong et al., 2011), indicating the importance of those LepR neurons with unknown transcription markers. For example, re-expression of LepRb in the Arc rescues obesity and diabetes from LepRb deficiency while deletion of LepRb in both POMC and AgRP neurons only produces mild obesity and no diabetes (de Luca et al., 2005; van de Wall et al., 2008). LepRb is also abundant in the DMH and the function of DMH LepRb received less attention until recently. DMH LepRb neurons receive prominent inputs from preoptic neurons and send strong projections to the brown adipose tissue (BAT) (Zhang et al., 2011b), indicating a role for these neurons in energy expenditure. Consistently, a role for these neurons in mediating leptin action on thermogenesis in diet-induced obesity mice has been demonstrated (Enriori et al., 2011). Taken together, these results suggest an importance for those neurons with unknown transcription markers in energy balance regulation; however, how these individual groups of neurons, which are loosely distributed in the hypothalamus, mediate leptin function is unclear. The neurotransmitters that mediate LepR neurons with unknown transcription markers remain largely unknown.

Given the importance of GABA and glutamate for neurotransmission in general, understanding the function of GABA and glutamate release from LepRb neurons will likely offer an opportunity to reveal the function of those LepRb neurons with unknown transcription markers. Notably, key hypothalamic regions with abundant LepRb expression are mainly glutamatergic or GABAergic. For GABA and glutamate to be released from synaptic terminals, they must first be packaged into vesicles via their specific vesicular transporters (Gasnier, 2004; Sudhof, 2004). Vesicular transporters use the proton electrochemical gradient, generated by vesicular H^+-ATPase, to drive neurotransmitters into the lumen of the vesicle. Global gene knockout of a vesicular transporter prevents release of the corresponding neurotransmitter from presynaptic terminals (Fremeau et al., 2004; Wojcik et al., 2004). For GABA (and glycine), there is only one vesicular GABA transporter (VGAT) (McIntire et al., 1997; Ebihara et al., 2003). For glutamate, there are 3 isoforms of vesicular glutamate transporters (VGLUTs), but only *Vglut2* is expressed in the hypothalamus (Tong et al., 2007). The Arc expresses abundant VGAT, but little VGLUTs (Fig. 2), suggesting the majority of Arc neurons are GABAergic. The VMH, PVH and PMv express abundant VGLUT2, but little VGAT, suggesting that neurons in these regions are largely glutamatergic (Fig. 2). The DMH expresses both VGLUT2 and VGAT, suggesting that the DMH is composed of both glutamatergic and GABAergic neurons (Fig. 2).

Using deletion of VGLUT2 or VGAT as a means to disrupt glutamate or GABA release, studies are beginning to reveal their roles in leptin neural pathways (Tong et al., 2007, 2008). It was widely speculated for a role of GABA co-released from AgRP neurons that compensates for AgRP and NPY, which results in lack of metabolic defects of AgRP and/or NPY

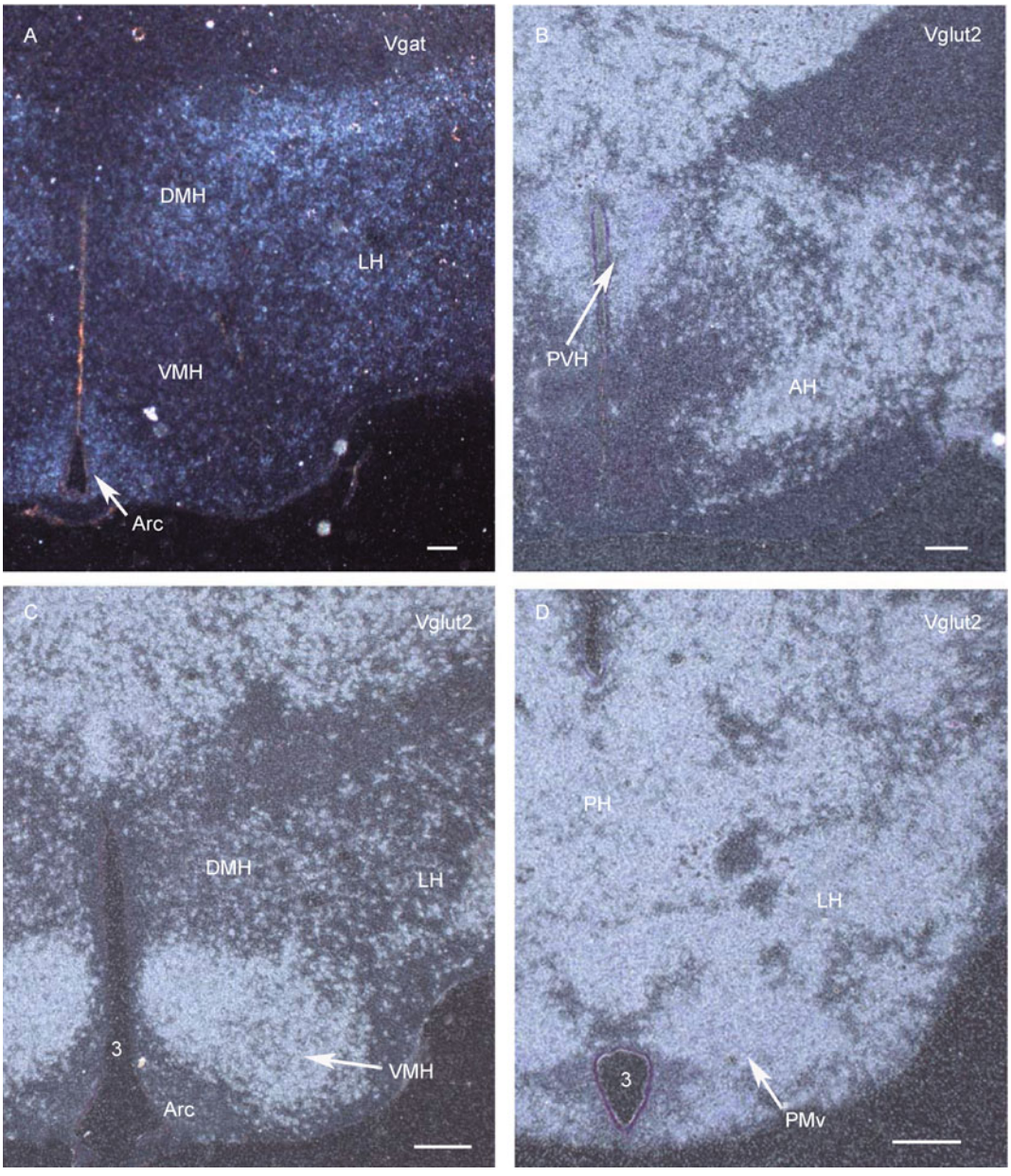

Figure 2. As shown by ^{35}S-labeled *in situ* hybridization histochemistry, *Vgat* and *Vglut2* mRNA display a complementary expression in the hypothalamus. (A) *Vgat* is expressed in the Arc, DMH, LH, and VMH. (B–D, from rostral to caudual) *Vglut2* is expressed in the AH, PVH and LH (B), DMH, VMH and LH (C), PH and PMv (D). AH: anterior hypothalamus; Arc: arcuate nucleus; DMH: dorsomedial hypothalamus; LH: lateral hypothalamus; PH: posterior hypothalamus; PMv: Ventral premammillary nucleus; VMH: vetromedial hypothalamus. Scale bars: 100 μmol/L.

deficiency (Flier, 2006). To specifically examine the function of GABA release from AgRP neurons, we have generated mice with ablation of GABA release from AgRP neurons and found that GABA release from these neurons restrains energy expenditure and increases body weight (Tong et al., 2008). Other studies based on lesion of AgRP neurons in adult mice suggested a powerful role of these neurons in promoting feeding, which involves GABA release (Wu et al., 2009). These results demonstrate the power of developmental compensation, which masks the effect of feeding defects in mice with ablation of GABA release from neonatal stages. The nature of developmental compensations remains elusive. Surprisingly, despite its strong expression in the VMH, VGLUT2 deletion in the VMH leads to normal body weight on chow and only slightly increased body weight on high fat diet, suggesting a role for other neurotransmitters in mediating body weight action of VMN neurons (Tong et al., 2007).

Notably, based on the animal model with LepRb deletion in

GABAergic neurons, a recent study revealed that LepRb in GABAergic neurons mediate a major part of leptin role on body weight regulation (Vong et al., 2011), suggesting an important role for GABA release in mediating leptin action. However, a direct test for the role of GABA in mediating leptin action is still lacking. Clearly, further studies are required to examine the role of glutamate release from the PVH and PMv, and glutamate and GABA release from the DMH.

NOVEL APPROACHES ARE EMERGING TO REDEFINE THE ROLE OF NEUROTRANSMITTERS

As discussed above, great advances have been achieved in our understanding of the neural pathways that mediate leptin function. The rapid progress is largely due to the emergence of new technologies including the Cre-loxP system which allows gene manipulations in specific groups of neurons. The growing number of mouse strains with neuron-specific expression of Cre recombinase in the hypothalamus and strains with genes flanked by loxP sites makes it possible to assign the function of the interested genes to specific groups of neurons (Gavériaux-Ruff and Kieffer, 2007). However, recent findings indicate that cautions need to be excised for data interpretation in those studies based on animal models with gene deletion in neonatal stages. First, it is difficult to differentiate the physiological function from developmental roles. For example, emerging data demonstrate a critical role for leptin in neuronal development (Bouret, 2010). In mice with deficiency of either leptin or leptin receptor, leptin-responsive neurons exhibit defects in axonal projections (Bouret et al., 2004a, 2004b), suggesting that leptin signaling pathways are important for normal development of these neurons. If this is true, then mouse models with defects in any steps within the leptin signaling pathways in one given group of neurons will have developmental defects in these neurons. Thus, the phenotype examined in the adult stage may not truly reflect the normal physiological function and may instead reflect the consequence of abnormal development. Second, emerging data suggest that developmental compensation can mask the true physiological function. For example, mice with AgRP neurons lesion in the adult stage exhibit severe hypophagia while those with AgRp neurons lesion in the neonatal stage are grossly normal (Luquet et al., 2005). These data demonstrate the existence of compensatory pathways that efficiently mask the potential defects. Previous data show that administration of both AgRP and NPY produces salient orexigenic effects while mice with deletion of these neuropeptides exhibit a normal phenotype (Qian et al., 2002; Kalra and Kalra, 2004), suggesting that developmental compensations also occur in response to defective neurotransmitter release.

To circumvent potential complications in data interpretation, novel techniques have been emerging to specifically reveal the physiology in the adult stage. One such approach is to delete gene of interest at the adult stage using inducible expression of Cre recombinase based on Cre-loxP technology (Garcia and Mills, 2002). Although this approach has been widely used for other brain areas (Erdmann et al., 2007; Stubbusch et al., 2011), studies using this approach on hypothalamic neurons for energy balance regulation remain sparse. Nonetheless, a Cre-inducible mouse strain for gonadotrophin-releasing hormone neurons has been reported (Wolfe et al., 2008). The second approach is the newly developed optogenetics. This technique is based on expression of light-sensitive microbial opsins, which themselves are non-selective cation channels, in selective subsets of neurons, and these neurons can then be activated by applying light (Fenno et al., 2011). This technique has proven to be powerful in the dissection of neurocircuits regulating behavior (Deisseroth, 2011; Diester et al., 2011). Within the hypothalamus, this approach has been used to study the role of orexin neurons in sleep-wake regulation and AgRP neurons in feeding (Adamantidis et al., 2010; Aponte et al., 2011). Light-stimulation of AgRP neurons rapidly induces feeding behavior in a reversible manner, underscoring the salient role of AgRP neurons in feeding regulation (Aponte et al., 2011). The third approach is the development of designer receptor exclusively activated by designer drugs (DREADD) technology. This technology involves expression of mutated muscarinic G-protein-coupled receptors (GPCRs), which lose the ability to bind natural ligands, but gain the ability to bind the otherwise pharmacologically inert ligand clozapine-N-oxide (CNO) (Alexander et al., 2009; Ferguson et al., 2011). Dependent on different versions of mutant GPCRs used, CNO activation can induce either excitation or inhibition of selective groups of neurons with expression of these GPCRs (Alexander et al., 2009; Ferguson et al., 2011). The proof of concept experiment for this technique has been performed on AgRP neurons, which upon activated or inhibited by CNO, rapidly and reversibly induce or inhibit feeding (Krashes et al., 2011), proving the usefulness of this technique in unraveling the circuitry controlling feeding and its related behaviors.

In consideration of using these novel techniques for studying neurotransmitters in body weight regulation, the first approach with inducible gene deletion will be instrumental to understand physiological function of neurotransmitters through deletion of the gene encoding either neuropeptides or proteins required for the release of fast-acting neurotransmitters at the adult stage. For the latter two approaches, since neurons will be activated or inhibited, and accompanying changes in neuronal activity will ultimately alter the release of all neurotransmitters from the neurons, one might anticipate difficulties in using these approaches to study the function of individual neurotransmitters. However, an approach using the combination of inducible neuron activation and concurrent loss of neurotransmitter release should be powerful to reveal the role of the neurotransmitter in mediating the function of

neurons. For example, mice can be generated in which AgRP neurons express either light-activatable opsin or CNO-activatable DREADD, and concurrently lack AgRP, NPY or GABA release. Using these mice, specific requirement of the individual transmitters in mediating AgRP action on feeding will be precisely revealed through inducible activation of AgRP neurons by light or CNO.

PERSPECTIVE

In summary, exciting progress has been made in our understanding of neurotransmitters with the hypothalamic neural pathway regulating body weight. It is well established that leptin action in the hypothalamus is critical for body weight homeostasis. However, the vast majority of obesity is associated with high leptin levels, indicative of leptin resistance. Thus, strategies aiming at reversing leptin resistance or activating post-leptin resistance signal steps, bypassing leptin resistance, represent promising therapeutics for obesity treatment. A large body of evidence suggests multiple molecules and intracellular leptin signaling pathways as leptin resistance sites (Myers et al., 2010). Neurotransmitter release and its action on postsynaptic neurons represent major post-leptin resistance steps. With the advent of new technologies, more neurotransmitters and new function of existing neurotransmitters will be identified, which will lead to delineation of brain mechanisms governing energy balance and provide novel targets for specific and effective drugs to reverse and prevent the current devastating obesity epidemic.

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ABBREVIATIONS

ACTH, adrenocorticotropin; AH: anterior hypothalamus; Arc: the arcuate nucleus; AgRP, agouti-gene related protein; BDNF, brain-derived neurothophic factor; BMI, body mass index; CART, cocaine- and amphetamine-regulated transcript peptide; CNO, clozapine-N-oxide; CRH, corticotrophin releasing hormone; DMH: dorsomedial hypothalamus; DREADD, designer receptor exclusively activated by designer drugs; GALP, Galanin-like peptide;GPCRs, G-protein-coupled receptors; LepRb, B isoform of leptin receptors; LH: lateral hypothalamus; f: fornix; 3V: the third ventricle; MCH, melanin-concentrating hormones; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating polypeptide; PH: posterior hypothalamus; PMv: Ventral premammillary nucleus; POMC, proopiomelancortin; PVH, paraventricular hypothalamus; SF1, steroidogenic factor 1; TRH, thyrotropin releasing hormone; UCP, uncoupling protein; VGAT, vesicular GABA transporter; VGLUTs, vesicular glutamate transporters; VMH, ventromedial hypothalamus

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