Chapter IX

HYPOTHALAMIC CONTROL OF APPETITE AND ENERGY METABOLISM

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Introduction

The hypothalamus is a critical brain structure important for autonomic function, including the regulation of energy metabolism, glucose metabolism, appetite and adiposity. This hypothalamic control of energy metabolism is a highly complex system that involves the central sensation, coordination and integration of peripherally derived nutrients and hormones. These central hypothalamic processes are then translated into neurochemical code, conveyed through the autonomic nervous system (ANS), to innervate peripheral target tissues. In this manner, negative feedback from the periphery to the hypothalamus maintains energy metabolism in equilibrium, i.e. energy intake is matched to energy expenditure. In terms of energy metabolism, a disturbance in negative feedback from the periphery to the central nervous system (CNS) can cause significant metabolic disease such as obesity and diabetes. This chapter focuses on the hypothalamic control of appetite, with particular focus on neuroendocrine feedback regulation of energy and glucose metabolism. Finally, this chapter examines how dysfunctional hypothalamic circuits contribute to metabolic disease such as obesity and diabetes.

Historical Overview

An important role for the hypothalamus in energy metabolism was first discovered over 100 years ago, when it was noted that hypothalamic injury, but not pituitary injury resulted in marked obesity. Hetherington and Ranson demonstrated conclusively that this marked obesity was due to electrolytic lesions of the hypothalamus (Hetherington and Ranson, 1940). In this study, marked obesity developed from extensive bilateral damage (not unilateral damage) to
the dorsomedial hypothalamic (DMH) and ventromedial hypothalamic (VMH) nuclei, the arcuate nucleus (ARC), the fornix, the medial portion of the lateral hypothalamus and the ventral premammillary nucleus. These authors did not define the VMH nucleus as a the key hypothalamic site, but rather noted that “Symmetrical destruction of the ventral portion of this area, including the nuclei and possibly other structures near the base, seems to be more important than injury to the more dorsal structures for the production of maximum adiposity” (Hetherington and Ranson, 1940). In 1942, Hetherington and Ranson described most pronounced obesity with ‘bilateral anterior tuberal lesion’, which consist of lesions to three quarters of the VMH, or ‘bilateral tubero-premammillary lesion’, which consist of lesions to the caudal third of the VMH and premammillary area. The authors concluded that “Obesity can be produced by fairly symmetrical lesions which destroy bilaterally: a) most of the VMH together with some of the tissue immediately around them, especially on their lateral sides; 2) the caudal ends of the VMH, the premammillary area, and a considerable part of the lateral hypothalamic areas adjacent to it”. Brobeck et al validated these data in 1943, in which the authors showed voracious hyperphagia due to lesions of the VMH (Brobeck et al., 1943). These studies are the first to establish that the VMH functions normally as a “satiety center” (Figure 1).

Although the presence of a hypothalamic “feeding center” had been previously hypothesized by Clarke in 1939, this area wasn’t discovered until 1951 when Anand and Brobeck showed that bilateral destruction of the lateral hypothalamus completely suppressed spontaneous eating. Again, unilateral lesion to the lateral hypothalamus had no effect on feeding behavior suggesting significant compensatory plasticity in the non-lesion side. Furthermore, hyperphagia and obesity in rats with VMH lesions could be completely reversed when the same rats had a second lateral hypothalamic lesion to the ‘feeding center’ (Anand and Brobeck, 1951; Figure 1). This is the first evidence that hypothalamic neuronal circuits controlling appetite act in a hierarchical manner, and suggests that the brain is wired to prevent starvation, rather than prevent overeating. Despite the development of novel molecular genetic technologies, these earlier observations still generally hold true. The VMH ‘satiety centre’ is a major site of feeding inhibition, through the direct actions of leptin on leptin receptor-containing VMH neurons (Dhillon et al., 2006). The lateral hypothalamus “feeding center” houses both appetite-stimulating orexin and melanin concentrating hormone (MCH) neurons. Further, this nucleus plays a critical role in integrating and relaying information from reward pathways in midbrain dopaminergic systems and homeostatic appetite regulation via the melanocortin system.

The discovery of the melanocortin system was initially missed by early lesion studies and is the missing piece of the hypothalamic puzzle in the control of appetite. The melanocortin system comprises ARC neuropeptide Y (NPY)/agouti related peptide (AgRP) and proopiomelanocortin (POMC) neurons and their projections to the paraventricular nucleus (PVN). The critical role of these nuclei in the hypothalamic control of appetite will be detailed in subsequent sections.
Figure 1. A summary of early lesion studies illustrates the importance of hypothalamic nuclei in the control of appetite and body weight. A, Cresyl violet stained section showing the location of the lateral hypothalamus (LH), arcuate nucleus (ARC), the ventromedial hypothalamic nucleus (VMH) and the dorsomedial hypothalamic nucleus (DMH). B, Graphical illustration of the nuclei shown in A. C, Bilateral, not unilateral, lesions of the VMH caused obesity indicating that the VMH normally acts as a satiety center. D, Bilateral, but not unilateral lesions, of the LH caused starvation indicating the normal function of the LH promotes appetite. E, Bilateral lesions of the VMH caused obesity. However, subsequent lesions of the lateral hypothalamus in the same animal reversed the obese phenotype and caused starvation. This is the first evidence that hypothalamic neuronal circuits controlling appetite act in a hierarchical manner, and suggests that the brain is wired in such way to prevent starvation, rather than prevent overeating.
Early studies suggested that humoral factors from peripheral tissues affect the hypothalamic control of appetite. For example, denervation of the gastro-intestinal tract did not inhibit feeding, suggesting that feeding can occur normally without sensation from the stomach and intestine (Janowitz and Grossman, 1949). From early experiments researchers realized that signals from the body must regulate the hypothalamic control of feeding. This lead to Mayer’s proposal of the glucostatic theory in 1953, in which increased blood glucose after a meal drives satiety (Mayer, 1953). In 1950, Kennedy proposed the lipostatic hypothesis, in which feeding is regulated by “the concentration of certain metabolites, as yet unspecified” (Kennedy, 1950). The nature of the lipostatic signal emanating from the adipose tissue took another 44 years to be discovered. The discovery of leptin in adipose tissue in 1994 by Friedman and colleagues caused an explosion of interest in the hypothalamic regulation of appetite and metabolism (Y. Zhang et al., 1994). However, the discovery of leptin was based on pioneering work by Coleman and colleagues using parabiotic pairs of mice (Coleman and Hummel, 1969). Parabiosis refers to a skin-to-skin anastomosis formed by surgical joining two mice from the shoulder to the pelvic girdle. Within 3-4 days, after the wound has healed, the parabiotic pairs share a common blood supply. Coleman hypothesized that the obesity observed in mutant db/db mice was caused by a circulating factor and that this factor would cause obesity in a normal mouse sharing a common parabiotic blood supply. Although Coleman’s hypothesis was wrong, the results were nonetheless remarkable. The normal mouse in this parabiotic pair died of starvation. Coleman discovered that the db/db mouse produced a satiety factor so powerful that it drove the normal mouse to starvation (Coleman and Hummel, 1969). A parabiotic pairing of the obese ob/ob mouse and a normal mouse reduced weight gain and food intake in the ob/ob mouse, however the paired mice lived for months until the end of the experiment (Coleman, 1973), suggesting the normal mouse produced the same satiety factor as the db/db mouse but at insufficient amounts to cause starvation. Parabiosis of the ob/ob mouse with the db/db mouse allowed Coleman to make his profound conclusions. In this pairing, the ob/ob eventually starved to death after 20-30 days while the db/db mouse gorged on food and gained weight at normal rates (Coleman, 1973). Coleman had demonstrated that the db/db mouse produced a satiety factor but did not respond to it, whereas the ob/ob mouse responded to the factor but did not produce it (Figure 2). Further, lesion studies showed that the receptor for the satiety factor was found in the VMH and ARC (Coleman and Hummel, 1970). The cloning of leptin and the leptin receptor essentially verified all of Coleman’s predictions and highlighted a classic neuroendocrine feedback loop that controls metabolism (Elmquist et al., 1998; Gautron and Elmquist, 2011). Leptin, produced by the ob/ob gene in the adipose tissue, enters the blood and activates leptin receptors, encoded by the db/db gene, in the hypothalamus to reduce food intake and activate energy expenditure. A reduction in adipose tissue reduces leptin in the circulation and the entire system is held in check; at a common set point. Many additional endocrine factors have subsequently been discovered to regulate appetite and energy metabolism through feedback mechanisms, including ghrelin, peptide YY (PYY) and glucagon like peptide 1. This chapter will examine new developments in the hypothalamic control of appetite and energy metabolism.
Figure 2. A summary of parabiosis experiments proving the hypothesis that a circulating factor in the blood suppresses food intake. These critical studies influenced subsequent studies and helped to lead to the discovery of leptin. A, A parabiotic pairing of obese and diabetic db/db mice with normal lean mice caused starvation in the lean mouse, whereas the db/db mouse continued to eat normally. This shows that the db/db mouse overproduces a satiety factor but does not respond to it. The lean mouse responds to the overproduced satiety factor and starves. B, A parabiotic pairing of obese db/db mice with obese ob/ob caused starvation in the ob/ob, whereas the db/db mouse continued to eat normally. This shows that ob/ob mice are obese because they do not produce the satiety factor but respond to overproduction of the satiety factor in db/db mice and starve. C, This pairing showed that normal lean mice also produced the satiety factor but that production of the satiety factor in lean wt mice is not sufficient to cause starvation in ob/ob mice. This demonstrated that the circulating satiety factor, later to be discovered as leptin, is produced proportional to fat mass.
The Hypothalamic Nuclei Controlling Appetite

ARC

Although initial experiments by Hetherington and Anand showed that the VMH and lateral hypothalamus are key appetite regulating centers in the hypothalamus, the ARC has received the most attention over the last 15 years and as arguably the most critical hypothalamic nucleus. There are two key appetite-regulating neuronal populations in the ARC. NPY and AgRP are co-expressed in neurons of the ARC and are potent orexigenic peptides, whereas the POMC precursor protein is cleaved into potent anorexigenic α-melanocyte-stimulating hormone (α-MSH) and peptides (Figure 3). Because AgRP is only expressed in the ARC nucleus, this chapter will refer to NPY/AgRP only as AgRP neurons. AgRP and POMC neurons in the ARC are arguably considered “first-order” sensory neurons in the control of food intake and receive, coordinate and respond to changes in metabolic status. Both AgRP and POMC neurons project to the PVN, where the anorectic effects of α-MSH peptides are mediated by melanocortin 4 receptors (MC4R). NPY Y1 and Y5 receptors in the PVN mediate the orexigenic effects of NPY, whereas AgRP antagonizes the effect of α-MSH on the MC4R (Figure 4). This system is collectively known as the melanocortin system (Figure 3). A unique feature of the melanocortin system is the ability of AgRP neurons to suppress POMC cell firing via inhibitory GABAergic inputs (Andrews et al., 2008; Cowley et al., 2003). There is no evidence that POMC neurons feed back to inhibit AgRP neuronal firing despite the expression of GABA in POMC neurons (Hentges et al., 2004; Hentges et al., 2009). This anatomical arrangement of the melanocortin system provides one simple mechanism through which the hypothalamus is geared to promote food intake, rather than satiety. For instance, activation of AgRP neurons directly increases food intake while simultaneously inhibiting satiety, analogous to driving a car, in which maximal speed is achieved by releasing the brake and pressing the accelerator. From an evolutionary standpoint, this melanocortin circuitry maintains a hunger stimulus during periods of food scarcity and promotes food intake to ensure survival. However, in today’s energy-abundant society, this circuitry contributes to the obesity epidemic by increasing hyperphagia.

The hypothalamic melanocortin circuits in the ARC and PVN have been extensive studied, but it should be noted that POMC and AgRP neurons project to other regions of the brain that are important for appetite regulation, including the medial preoptic area (MPA), the nucleus of the solitary tract (NTS) and the parabrachial nucleus (PBN) (Broberger et al., 1998; Elias et al., 1998). Understanding how these melanocortin circuits influence appetite regulation in these distal target areas will be important future directions. Indeed, recent research shows that GABAergic transmission in AgRP neurons innervating the PBN is critical to prevent starvation (Wu et al., 2009; see below).

POMC Neurons Controlling Food Intake and Body Weight

The critical importance of POMC neurons in appetite and energy balance is highlighted by elegant conditional gene ablation experiments. Ablation of POMC neurons in adulthood produced an increase in food intake and body weight (Gropp et al., 2005). Because ablation of
Figure 3. The melanocortin system (ARC-PVN) circuits controlling food intake and body weight regulation. The arcuate nucleus (ARC) houses neurons that coexpress NPY (blue), AgRP (red) and GABA (purple). These neurons stimulate food intake by acting at downstream receptors in the paraventricular nucleus (PVN). The ARC nucleus also houses a population of POMC (orange) neurons that produce the anorectic alpha melanostimulating hormone ($\alpha$-MSH) peptide. Increased activity of POMC neurons elevates $\alpha$MSH in the PVN, which in turn acts on melanocortin 4 receptor (MC4R)-containing neurons in the PVN to suppress food intake. NPY acts on Y1 and Y5 receptors in the PVN to stimulate food intake, whereas AgRP antagonizes MC4R and prevents the anorectic actions of $\alpha$MSH. Currently there is some debate whether AgRP is an antagonist or an inverse agonist at the MC4R. Efferent outputs from the PVN project to numerous areas in the brain and brainstem to coordinate feeding behaviour, energy expenditure and adiposity. GABA is also an important neurotransmitter secreted from NPY/AgRP neurons in the regulation of food intake. Inhibitory GABA inputs from NPY/AgRP neurons synapse onto POMC neurons within the ARC to suppress the anorectic effects of $\alpha$MSH secreted from POMC neurons. Recent studies show that GABA maintains hypothalamic orexigenic tone, as mice engineered to prevent GABA release from NPY/AgRP neurons show a lean anorectic phenotype. NPY neurons respond to circulating hormones and contain many receptor hormones including the ghrelin receptor (GHSR), the leptin receptor (INSR) and the leptin receptor (ObR). 3V, third ventricle.
Figure 4. Interaction between AgRP and α-MSH at distal melanocortin 4 receptor (MC4R) sites in the PVN. POMC neurons produce α-MSH that acts on MC4Rs in the PVN to suppress food intake. Increased activity of AgRP neurons causes the release of AgRP peptide from nerve terminals in the PVN. AgRP binds to the MC4R and antagonises the effect of α-MSH at the MC4R. By preventing the actions of α-MSH in the PVN, AgRP helps to increase food intake. AgRP neurons also produce NPY, however NPY acts on NPY Y1 and Y5 receptors in the PVN independently from the MC4R.
POMC neurons leads to obesity, many recent studies have focused on the hormonal and molecular mechanisms regulating POMC neuronal function and the physiological consequence of perturbations to POMC neurons. Seminal studies show that POMC neurons regulate the hypophagic actions of leptin signaling in the brain (Balthasar et al., 2004), although not exclusively as VMH neurons are also important (Bingham et al., 2008; Dhillon et al., 2006; see below). Activation of the leptin receptor on POMC neurons initiates hypophagia through the janus kinase (JAK) - signal-transducer activator of transcription (STAT) 3 pathway (Balthasar et al., 2004; Bates et al., 2003; Munzberg et al., 2003). Leptin binds to its receptor and autophosphorylates JAK2 that then recruits and phosphorylates STAT3 (Baumann et al., 1996). pSTAT3 dimerizes and enters the nucleus where it binds to specific DNA elements in the POMC or AgRP promoter to activate POMC and repress AgRP gene expression respectively (Bates et al., 2003; Figure 5). Indeed, nuclear pSTAT3 immunostaining is extensively used as a marker of leptin sensitive neurons in the brain. There is a wealth of literature showing that leptin is a major regulator of POMC. Leptin-deficiency or leptin receptor deficiency reduces POMC gene expression and leptin replacement to ob/ob mice increases POMC (Korner et al., 1999; Mizuno et al., 1998). Moreover, fasting reduces leptin levels and also reduces POMC gene expression (Korner et al., 1999; Mizuno et al., 1998). After activating the STAT3 pathway, leptin induces suppressor of cytokine signaling 3 (SOCS3), which acts as a feedback inhibitor of leptin receptor signaling (Elias et al., 1999). Dynamic control of the STAT3 pathway is required for ongoing leptin sensitivity in POMC neurons. For example, the non-tyrosine phosphatase SHP2 helps to dephosphorylate the STAT3 pathway and maintain leptin sensitivity, whereas dephosphorylation of pSTAT3 by the protein tyrosine phosphatase PTPB1, reduces POMC leptin sensitivity (Banno et al., 2010). However, POMC deletion of STAT3 results in only a slight increase in body weight and food intake (Xu et al., 2007) suggesting other signaling pathways in POMC neurons are involved in leptin’s anorectic action.

In addition to the STAT3 pathway, leptin can also utilize the phosphatidylinositol 3-kinase (PI3K) pathway within POMC cells. PI3K phosphorylates the membrane lipidphosphatidylinositol-4,5-bisphosphate (PIP2) after activation to form phosphatidylinositol-3,4,5-trisphosphate (PIP3). The tumour suppressor PTEN (phosphatase and tensin homologue) is a lipid phosphatase that dephosphorylates PIP3 to PIP2. Accumulation of PIP3 recruits several kinases to the plasma membrane, which propagates further signaling cascades. Leptin directly activates PI3K signaling in POMC neurons (Xu et al., 2005) and inhibiting the PI3K pathway attenuates both the anorectic effects of insulin and leptin (Niswender et al., 2001; Niswender and Schwartz, 2003). Genetic deletion of PI3K subunits in POMC neurons reduced acute feeding responses to leptin and attenuated leptin-induced action potential firing in POMC neurons (Hill et al., 2008). Despite preventing the acute actions of leptin, genetic deletion of PI3K in POMC neurons had no effect on long-term body weight regulation.

When all the data is considered together, it is clear that leptin activates POMC neurons, either via the STAT3 or the PI3K pathway, to regulate feeding and body weight. However, gene deletion studies in POMC neurons show that other neurons and hypothalamic nuclei are critical to reproduce the full metabolic phenotype seen in leptin-receptor deficient mice. Because deletion of leptin receptors on POMC neurons does not fully recapitulate the obesity seen in db/db leptin receptor-deficient mice, a recent study examined whether leptin-receptor containing neurons regulate POMC neurons presynaptically. In this study, leptin receptors
Figure 5. Leptin and insulin intracellular signaling mechanisms in hypothalamic neurons. Activation of the leptin receptor initiates the janus kinase (JAK) - signal-transducer activator of transcription (STAT) 3 pathway. Leptin binds to its receptor and autophosphorylates JAK2 that then recruits and phosphorylates STAT3. pSTAT3 dimerizes and enters the nucleus where it binds to specific DNA elements in the POMC or AgRP promoter to activate POMC and repress AgRP gene expression respectively. After activating the STAT3 pathway, leptin induces suppressor of cytokine signaling 3 (SOCS3), which acts as a feedback inhibitor of leptin receptor signaling. SHP2 helps to dephosphorylate the STAT3 pathway and maintain leptin sensitivity, whereas dephosphorylation of pSTAT3 by the protein tyrosine phosphatase PTPB1, reduces POMC leptin sensitivity. SOC3 inhibits the actions of SHP2 to repress the pSTAT3 pathway. It remains unknown, as to whether SOCS activates PTPB1 to further repress leptin signaling through the pSTAT3 pathway. Leptin also activates the PI3K pathway, however the cellular response to leptin-activated PI3K appears neuron specific. For example, leptin depolarizes POMC neurons through PI3K and non-specific cation channel activation (TRP) [as shown in pathway a], however leptin hyperpolarizes AgRP neurons by PI3K-mediated opening of KATP channels and subsequent potassium outflow [shown in pathway b]. Insulin hyperpolarizes both POMC and AgRP neurons by PI3K-mediated activation of KATP channels. Downstream of PI3K, insulin and leptin both stimulate the phosphorylation and nuclear exclusion of FOXO1. Preventing FOXO1 from entering the nucleus suppresses AgRP transcription and enables POMC transcription, an essential enzyme in posttranslational processing of α-MSH. The increase in POMC and α-MSH increases the inhibitory tone in the melanocortin system.

were deleted from GABAergic or glutamatergic neurons and the affect on hyperphagia and obesity observed (Vong et al., 2011). The results were remarkable and showed that deleting leptin receptors from glutamatergic neurons had little or no effect on obesity, whereas
deleting leptin receptors on GABAergic neurons caused marked obesity, similar to that seen in leptin receptor deficient db/db mice. Further analysis showed that leptin suppressed presynaptic GABAergic inhibitory inputs to POMC neurons, which subsequently disinhibited POMC neuronal firing and increased satiety (Figure 6). These leptin receptor neurons that synapse with POMC neurons are found in the ARC (majority of which are not AgRP neurons), DMH and lateral hypothalamus. This study suggests that although leptin acts directly on POMC neurons it also regulates POMC neurons indirectly by acting on upstream presynaptic GABAergic, and not glutamatergic, neurons. Moreover, the genetic deletion of leptin receptors from GABAergic neurons, rather than from POMC neurons, more closely resembles the obese phenotype of leptin receptor deficient mice. Thus, presynaptic regulation of POMC neuronal firing is essential to maintain normal energy metabolism.

In addition to leptin, insulin targets POMC neurons and influences food intake and energy metabolism. POMC neurons express both insulin and leptin receptors, although a recent study suggests that two distinct subpopulations of POMC neurons exist; those that respond to insulin and those that respond to leptin (Williams et al., 2010). Indeed, insulin directly activates PI3K signaling in POMC neurons and inhibiting PI3K blocks the anorectic effects of insulin (Xu et al., 2005). However, recent studies have questioned the acute anorectic role of insulin in rats (Jessen et al., 2010; Tups et al., 2010), or suggest that the anorectic actions of insulin require simultaneous leptin receptor activation (Tups et al., 2010), possibly due to enhanced intracellular signaling through STAT3 and PI3K pathway crosstalk (Figure 5), as was recently described to regulate glucose homeostasis (Koch et al., 2010). Moreover, genetic deletion of the insulin receptor in POMC neurons did not affect long term body weight, glucose homeostasis or food intake (Konner et al., 2007), suggesting neurons other than POMC are required for insulin’s ability to control energy homeostasis. Although leptin and insulin both appear to have anorectic actions of food intake, only genetic deletion of the leptin receptor on POMC neurons causes obesity (Balthasar et al., 2004; Hill et al., 2010; Konner et al., 2007). Moreover, when both the insulin receptor and the leptin receptor are deleted in POMC neurons, the obesity observed in the leptin receptor-deficient POMC neurons is completely reversed. This suggests that insulin and leptin have different functions in body weight regulation. Recent studies demonstrated that nicotine activates POMC neurons and attenuates food intake, providing strong biological evidence that links cigarette smoking to lower body weights (Mineur et al., 2011).

**POMC Neurons Regulate Whole Body Glucose Homeostasis**

POMC neurons not only affect energy metabolism by reducing food intake, but also play a salient role in maintaining whole body glucose homeostasis. Studies show that impaired glucose sensing in POMC neurons leads to peripheral glucose intolerance and insulin resistance (Parton et al., 2007) via a brain-liver circuit involving hypothalamic neurons and vagal autonomic innervation of liver (Yi et al., 2010). This effect involves the coordination and integration of multiple hormonal inputs on POMC neurons. A recent elegant study illustrated that both insulin and leptin receptor signaling in POMC neurons is required for normal glucose homeostasis (Hill et al., 2010). In this study, single deletion of the insulin receptor or the leptin receptor on POMC had no effect on glucose homeostasis, including...
Figure 6. The neuroendocrine regulation of hypothalamic appetite circuits. Within the hypothalamus there are many important nuclei involved in the control of appetite, including the ARC, VMH, LH, DMH and the PVN. The PVN is not shown here as it’s role in appetite is detailed in Figure 3 and Figure 4. As shown in the diagram, the circuits controlling appetite are complicated and interconnected. The ARC is a key nucleus that houses AgRP and POMC neurons. These neurons integrate hormonal information and receive and send numerous inputs and outputs within the hypothalamus.

Ghrelin increases appetite by activating AgRP neurons, which subsequently inhibit POMC neurons via inhibitory GABAergic inputs. Both leptin and insulin inhibit AgRP neurons. These AgRP neurons also communicate with orexin and melanin concentrating hormone (MCH) neurons in the lateral hypothalamus. Ghrelin receptors are present in the VMH and DMH although the function of the receptor in these nuclei remains unknown. POMC neurons in the ARC suppress appetite and are directly activated by leptin and inhibited by insulin. Recent studies show that leptin-responsive GABAergic neurons are found in the DMH, LH and ARC and that these leptin-responsive neurons play a more important anti-obesity role than leptin receptors directly on POMC neurons. POMC neurons can influence the activity of both orexin and MCH in the lateral hypothalamus and orexin neurons synapse on POMC neurons, presumably to reduce POMC activity and suppress appetite. This may be one mechanism, through which the VMH induces satiety and why lesions of the VMH result in obesity, as described over 60 years ago. Leptin also activates neurons in the VMH to suppress appetite, presumably by increasing the excitatory glutamatergic drive to POMC neurons or by increasing BDNF signaling in the VMH. Although leptin increases BDNF in the VMH, the mechanism through which BDNF prevents obesity does not involve the melanocortin system and remains unknown. BDNF in the DMH also prevents obesity, however it is not known if leptin activates BDNF in the DMH. Interestingly, insulin signalling in the VMH promotes diet-induced obesity. Insulin activates PI3K signalling and reduces VMH insulin receptor neuronal firing, which in turn reduces the excitatory drive on to POMC neurons and restricts satiety in DIO. There is a population of NPY neurons in the DMH that also increase appetite. These neurons represent a transient population that appears during periods of negative energy balance such as prolonged fasting, calorie restriction or lactation. The DMH contains a population of leptin receptor GABA neurons, which inhibit POMC neuronal firing in the ARC and suppress appetite. In the lateral hypothalamus orexin neurons stimulate food intake by influencing the melanocortin system in the ARC and also by enhancing the rewarding properties of food in the VTA. Leptin receptors are not present on orexin or MCH neurons but leptin receptor GABA neurons synapse with orexin neurons and POMC neurons, but not MCH neurons, to suppress appetite. While leptin plays an important role on neurons in the lateral hypothalamus, ghrelin does not appear to play a direct role as the ghrelin receptor is not present in the lateral hypothalamus. Little is known about how insulin affects neurons in the lateral hypothalamus.
plasma insulin concentration, hepatic glucose production, peripheral glucose disposal, glucose infusion rate during euglycemic clamps, glucose tolerance tests and insulin tolerance tests, in accord with a previously published paper (Konner et al., 2007). However, deletion of both insulin and leptin receptors on POMC neurons caused insulin resistance and glucose intolerance independent of changes in body weight. These results show a level of functional redundancy in insulin and leptin actions on POMC neurons in terms of glucose homeostasis, as deleting both insulin and leptin receptors prevents the compensatory change. Importantly, these results highlight the need to examine how hormones and nutrients interact to affect not just POMC function, but also other important neurons that control energy homeostasis. The fact that impaired glucose tolerance occurs independent from changes in body weight and food intake, highlights the complexity of signaling mechanisms within POMC neurons.

Although insulin and leptin receptors act together to maintain glucose homeostasis, the downstream molecular mechanisms remain ambiguous. POMC neurons produce α-MSH and activate MC4R receptors in the PVN. Central α-MSH injection can decrease basal insulin release (Banno et al., 2007) or increase insulin-stimulated glucose uptake and production (Heijboer et al., 2005; Obici et al., 2001). Further, MC4R antagonism failed to block hyperinsulinemia-induced inhibition of hepatic glucose production (Obici et al., 2002a; Obici et al., 2002b), but intracerebroventricular (icv) infusion of α-MSH stimulates glucose production via gluconeogenesis (Gutierrez-Juarez et al., 2004). These studies indicate α-MSH may act on non-MC4Rs in the hypothalamus to regulate glucose production. Future studies should think beyond the traditional confines of the ARC-PVN melanocortin circuits to help explain how POMC neurons control glucose homeostasis.

Agrp Neurons Regulate Food Intake and Body Weight

AgRP neurons in the ARC nucleus are critical for ghrelin-induced food intake as genetic ablation of AgRP (Luquet et al., 2007) or double-knockout of NPY and AgRP prevents ghrelin-induced food intake (Chen et al., 2004). Indeed, ghrelin induces feeding by robustly stimulating NPY and AgRP neuronal activity as assessed by electrophysiology (Andrews et al., 2008; Cowley et al., 2003) or fos immunoreactivity (Andrews et al., 2008; Cowley et al., 2003; Hewson and Dickson, 2000; Wang et al., 2002) and gene expression (Chen et al., 2004; Kamegai et al., 2000, 2001; Nakazato et al., 2001). Consistent with the effect of ghrelin on NPY and AgRP neuronal activity, the GHSR1a is expressed on >90% of all NPY neurons in the ARC (Willesen et al., 1999). However, the GHSR1a is only expressed on less than 8% of POMC neurons (Willesen et al., 1999).

Despite the well-described effects of ghrelin on AgRP neurons in the ARC, neither NPY nor AgRP single gene deletion (Erickson et al., 1996), nor NPY and AgRP double knockout affected appetite and body weight (Qian et al., 2002). In order to rule out the development of compensatory mechanisms that could potentially explain the lack of effect in knockout models, two independent laboratories generated conditional AgRP neuronal ablation techniques. Conditional ablation of AgRP neurons during adulthood in the ARC, using the human diphtheria toxin targeted to the AgRP locus, results in a rapid reduction in food intake and body weight (Gropp et al., 2005; Luquet et al., 2005). In addition, Luquet et al (Luquet et al., 2005) showed that ablation of AgRP neurons during the early postnatal period did not
result anorexia and weight loss. These results argue that reorganization of the hypothalamic circuits during the developmental period can overcome AgRP neuronal ablation.

However, this conditional ablation approach is also not without technical limitations. For example, ablation of the AgRP neurons with diphtheria toxin destroys the neuron and the entire contents of the AgRP neuron, and as such the role of AgRP peptide is not tested, but rather the AgRP neuron with all neurotransmitters and neuropeptides. Recent studies show that GABA in AgRP neurons is the critical neurotransmitter affecting orexigenic pathways.

**GABA Signaling in the ARC**

GABA signaling in the brain influences appetite, and both AgRP neurons and POMC neurons in the ARC contain GABA (Hentges et al., 2004; Hentges et al., 2009; Horvath et al., 1997; Tong et al., 2008). Approximately 50-60% of all AgRP neurons express GABA (Horvath et al., 1997; Luquet et al., 2005; Wu et al., 2008), which led researchers to hypothesize that GABAergic neurotransmission in AgRP plays an important role in appetite. Initial observations illustrated a melanocortin-dependent mechanism whereby inhibitory GABAergic inputs from active AgRP neurons suppress POMC neuronal activity (Andrews et al., 2008; Cowley et al., 2003). GABA co-localizes with AgRP neurons that innervate POMC neurons in the ARC and distal target nuclei including the PVN (Horvath et al., 1997; Pu et al., 1999). Ghrelin activation of AgRP neurons increases GABAergic inhibitory postsynaptic currents and inhibitory synapses on POMC neurons (Andrews and Horvath, 2008; Andrews et al., 2008). Increased GABAergic inhibitory inputs on POMC neurons elevates food intake by lowering anorexigenic POMC neuronal activity. A recent study showed that deletion of vesicular GABA transporter in AgRP neurons, which prevents the synaptic release of GABA, removes the inhibitory tone onto postsynaptic POMC cells and produces a lean phenotype that is resistant to diet-induced obesity (Tong et al., 2008). Further, these mice have an attenuated hyperphagic response ghrelin (Tong et al., 2008). Although, GABA release from AgRP neurons suppresses POMC activity, Vong et al (Vong et al., 2011) showed that only 30% of all presynaptic GABAergic POMC inputs came from AgRP neurons, the remaining inputs came from non-AgRP GABA neurons in the ARC or from the DMH and lateral hypothalamus.

GABAergic AgRP neurons also have melanocortin-independent effects on appetite. Chronic blockade of the melanocortin pathway should lead to hyperphagia and obesity, as α-MSH cannot act on the MC4R to suppress food intake. However, ablation of AgRP neurons still causes starvation even in mice with chronic blockade of the melanocortin pathway (Wu et al., 2008). Wu et al discovered that GABA release from AgRP nerve terminals in the parabrachial nucleus (PBN) in the hindbrain is essential to maintain appetite. Although ablation of AgRP neurons caused starvation, this could be prevented by direct infusion of GABA_A agonists into the PBN. Moreover, infusion of the GABA_A antagonist, bicuculline, into the PBN inhibits feeding in a dose dependent manner and inactivation of GABA biosynthesis in the ARC causes anorexia. These results show that sudden loss of AgRP neurons prevents GABA signaling in the PBN and results in hyperactivity of a population of PBN neurons. The synaptic output of these hyperactive PBN neurons must act as a brake on an essential feeding circuit, which leads to starvation. Future studies are required to elucidate
the mechanisms underlying the function of the PBN in food intake. These studies clearly show that GABA signaling in hypothalamic AgRP neurons regulates appetite.

Approximately 40% of POMC neurons in the ARC nucleus also co-express GABA (Hentges et al., 2004; Hentges et al., 2009), although the direct effects of GABA signaling from POMC neurons on appetite and energy metabolism remain enigmatic. Another 25% of POMC neurons also express glutamate, raising the possibility that distinct subpopulations of POMC neurons control physiologically distinct roles in energy metabolism. Because GABA release from AgRP collateral projections in the ARC inhibit POMC neurons in the ARC, the question remains; do POMC neurons use GABA to directly inhibit AgRP neurons and thus create a complex short-loop feedback circuit in the ARC itself. Hentges et al. (Hentges et al., 2004) demonstrated that although ≈40% of POMC neurons co-express GABA in nerve terminal regions, little or no POMC neurons co-express GABA or vesicular glutamate in the ARC itself, suggesting that there is no GABAergic reciprocal feedback from POMC neurons to AgRP neurons. These recent studies that focus on GABA in AgRP neurons herald a new era in the central control of appetite. Although much of the work over the last 2 decades focuses on neuropeptides such as NPY, AgRP and POMC, the future advances in the field will elucidate how classic neurotransmitters such as GABA and glutamate affect neuronal circuits controlling appetite. Indeed, the recent demonstration that leptin receptor deletion on GABA, but not glutamatergic neurons (Vong et al., 2011), results in marked obesity exemplifies this notion.

Evolutionary Considerations of the Melanocortin System

Even though the conditional ablation of AgRP and POMC neurons destroys the entire neuron and not just the peptide of interest, these studies identify an intriguing evolutionary adaptation. AgRP neuron-ablated mice without any intervention starved to the point of death, whereas POMC neuron-ablated mice ‘only’ became obese (Gropp et al., 2005; Luquet et al., 2005). These results imply a greater evolutionary selection pressure for AgRP cell survival, via appropriate GABA transmission at innervation sites, compared to POMC cell survival in the ARC. AgRP activity is a signal of negative energy balance in the brain that promotes food intake to reestablish normal energy balance. On the other hand, POMC neurons respond to signals of positive energy balance, such as glucose, insulin and leptin, and help to reduce food intake and maintain normal energy balance. Given that our evolutionary history was almost completely dominated by periods of negative energy balance, it is not surprising that AgRP neurons developed different molecular mechanisms, compared to POMC neurons, to preserve cell function and appetitive drive. POMC neuronal activity suppresses food intake and therefore there is no adaptive evolutionary advantage to preserve POMC cell function. Without AgRP neurons in the ARC neurons starvation would occur, whereas removing POMC signaling ‘only’ causes obesity.

Hormonal Regulation of Agrp Neurons

Similar to POMC neurons, AgRP neurons receive hormonal input from the periphery including insulin, leptin and ghrelin. Leptin receptors are found on AgRP neurons in the ARC
(Draper et al., 2010) and leptin suppresses AgRP gene expression and transcription (Schwartz et al., 1998) through nuclear exclusion of FOXO1 (Fukuda et al., 2008; Kitamura et al., 2006) to restrict food intake. Leptin activates pSTAT3 and suppresses AgRP and NPY gene expression (Xu et al., 2007). Leptin receptor activation of AgRP neurons plays an important role in overall energy homeostasis as AgRP neurons lacking the leptin receptor displayed adiposity and increased body weight relative to controls (van de Wall et al., 2008). Deleting key intracellular signaling pathways had the same effect. Mice lacking STAT3 in AgRP were mildly hyperphagic and unresponsive to leptin. Consistent with this result, constitutively active STAT3 in AgRP neurons causes leanness and prevents high-fat diet-induced obesity due to increased locomotor activity and subsequent energy expenditure (Mesaros et al., 2008). No changes in food intake or gene expression were observed. Moreover, restoring leptin receptors in the ARC nucleus increased locomotor activity (Coppari et al., 2005). These studies show neuron-specific effects of leptin in the ARC. Leptin activates STAT3 signaling in POMC neurons to suppress appetite and activates STAT3 in AgRP neurons to increase energy expenditure. Collectively, these studies suggest that leptin-induced STAT3 signaling in AgRP neurons provides an unappreciated level of tonic inhibition on AgRP and subsequent food intake.

This idea is further supported by electrophysiological studies that show leptin hyperpolarizes AgRP neurons by activating ATP-sensitive potassium channels to inhibit action potential firing (Spanswick et al., 1997; van den Top et al., 2004). Further, leptin application suppresses PI3K signaling in AgRP neurons but leptin withdrawal from the slice preparation facilitates PI3K signaling in AgRP neurons, thereby mimicking low leptin conditions that increase AgRP expression, such as fasting (Xu et al., 2005). Finally, a recent study showed that leptin inhibits AgRP neuron firing through MAPK signaling and subsequent L-calcium current inhibition (Wang et al., 2008), linking neuronal firing properties to intracellular signal transduction.

Insulin is also an important regulator of AgRP neuronal function, as insulin inhibits AgRP gene expression (Schwartz et al., 1992), restricts action potential firing via ATP-dependent potassium channel (K<sub>ATP</sub>)-induced hyperpolarization in AgRP neurons (Konner et al., 2007) and suppresses food intake. The insulin receptor substrate is localized to AgRP neurons (Pardini et al., 2006). The effects of insulin on AgRP neurons are mediated predominantly through the phosphatidylinositol 3-OH-kinase (PI3K) (Xu et al., 2005). Brain-specific deletion of insulin receptors causes hyperphagia and susceptibility to diet-induced weight gain (Bruning et al., 2000), but despite the known effects of insulin on AgRP neurons, deletion of the insulin receptor on AgRP neurons, or POMC neurons, did not affect food intake or body weight (Konner et al., 2007). This illustrates that insulin receptor neurons, other than, or in addition to AgRP and POMC neurons are required for the anorectic effect of insulin on appetite and body weight.

Central insulin acts in the hypothalamus to robustly inhibit hepatic glucose production (Obici et al., 2002) via an insulin receptor action in the hypothalamus (Obici, Feng et al., 2002). This effect has been unequivocally ascribed to AgRP neurons as genetic deletion of the insulin receptor on AgRP neurons, but not POMC neurons, fails to suppress hepatic glucose production during euglycemic hyperinsulinemic clamp studies (Konner et al., 2007). It is important to keep in mind that central NPY injection increases glucose production and suppresses hepatic insulin sensitivity (Marks and Waite, 1997) and combining icv NPY injections with hyperinsulinemic clamps partially blocks the inhibitory effect of peripheral
hyperinsulinemia on hepatic glucose production. Moreover, denervation of the hepatic sympathetic nerves blocks the effect of central NPY on hepatic glucose production (van den Hoek et al., 2008). Thus, the inhibitory effect of insulin on hepatic glucose production requires an insulin-mediated suppression of AgRP neuronal activity and subsequent sympathetic nerve activity.

VMH

Early studies from the 1940s identified the VMH as a critical nucleus regulating appetite. Examination of the neuronal circuitry in the hypothalamus revealed that the VMH and ARC nucleus are reciprocally connected. For example the VMH receives afferent projections from the ARC (van den Hoek et al., 2008) and the ARC receives strong excitatory inputs from the VMH (Sternson et al., 2005). The VMH contains MC4R and NPY Y1, Y2, Y5 receptors and NPY infusions into the VMH increases feeding (Bouali et al., 1995; Harrold et al., 1999). Sternson et al, used laser scanning photostimulation to show that POMC neurons received strong excitatory input from the medial VMH, whereas AgRP neurons only received weak inhibitory input from within the ARC. Fasting diminished the strength of the excitatory input from the VMH to POMC neurons (Sternson et al., 2005). Further, the sensitivity of VMH neurons to α-MSH in food-deprived rats or rats pretreated with AgRP (Li and Davidowa, 2004) is suppressed, indicating that negative energy balance reduces the anorectic drive of VMH neurons onto POMC neurons. A recent study showed that deleting glutamate synaptic transmission from VMH neurons, increased long-term food intake and susceptibility to DIO (Tong et al., 2007), suggesting that the major excitatory output from the VMH is to suppress food intake, presumably acting on the POMC neurons, as described by Sternson et al (Sternson et al., 2005).

Unlike the neurons in the ARC nucleus, little is known about the chemical phenotype of VMH neurons that control appetite circuits. Nevertheless, brain-derived neurotrophic factor (BDNF) is one key protein as it is abundantly expressed in the VMH. Genetic deletion of BDNF or its receptor, TrkB, causes obesity in mice (Rios et al., 2001; Xu et al., 2003) and they are two of only a few obesity candidate genes in humans (Ramachandrappa and Farooqi, 2011). Fasting reduces leptin levels and suppresses BDNF gene expression specifically in the VMH, whereas leptin increases VMH BDNF gene expression, illustrating that BDNF is an important regulatory step in leptin signaling in the VMH. Indeed, deleting BDNF in the VMH and DMH produces hyperphagia and causes obesity (Unger et al., 2007). Importantly, the melanocortin system does not mediate the anorectic effects of BDNF, as BDNF still suppresses appetite and body weight in MC4R deficient mice (Xu et al., 2003). This indicates that BDNF may influence appetite and body weight through other means, such as classic neurotransmitter systems. Indeed, deleting glutamate release from VMH neurons results in obesity and hyperphagia (Tong et al., 2007), support the idea that BDNF may regulate glutamatergic neurotransmission in the VMH.

Leptin receptors are heavily expressed in the VMH (Elmquist et al., 1998), highlighting that this nucleus is highly sensitive to changes in hormone signaling and metabolic state. Leptin increases firing of VMH neurons and deleting the leptin receptor from VMH neurons, using the steroidogenic factor 1 (SF-1) cre mouse, causes hyperphagia, reduces energy
expenditure and predisposes mice to DIO (Bingham et al., 2008; Dhillon et al., 2006). These mice are also hyperinsulenic and glucose intolerant (Bingham et al., 2008; Dhillon et al., 2006), highlighting an important role for leptin signaling in VMH neurons in glucose homeostasis. SF-1 is a transcription factor exclusively expressed in the VMH and is required for the development of this nucleus. The generation of an SF-1 cre mouse permits the ability to knockout genes of interest only in the VMH to examine the function of this nucleus. These results help to define the targets through which leptin regulates appetite and body weight. In particular, leptin-receptor deficient db/db mice display severe obesity whereas leptin-receptor deficient POMC mice only display mild obese, suggesting leptin targets neuronal subpopulations other than POMC. Indeed, comparing the body weight phenotype of VMH and POMC leptin-receptor deficient mice, closely approximates the obese phenotype seen in leptin receptor db/db mice, suggesting these two circuits act independently but in parallel to maintain body weight homeostasis. SOCS3 is an inhibitor of leptin receptor JAK-STAT signaling and contributes to leptin resistance in DIO mice. Deletion of SOCS3 in VMH neurons pronounces JAK-STAT signaling through increased pSTAT3 levels and enhances sensitivity to peripherally injected leptin (Zhang et al., 2008). Furthermore, food intake was reduced in either chow-fed or high fat fed mice but body weight was not different due to compensatory reductions in energy expenditure. Despite no difference in body weight, mice lacking SOC3 in VMH neurons had improved glucose homeostasis and protected from hyperglycemia and hyperinsulinemia caused by DIO.

Because leptin activates PI3K signaling in POMC and inhibits PI3K signaling in AgRP neurons (Xu et al., 2005), and the VMH regulates the anorectic effects of leptin, Xu et al, deleted PI3K in VMH neurons (Xu et al., 2010) and examined the effects on energy homeostasis. These mice were susceptible to DIO, had impaired energy expenditure and showed a blunted response to leptin. Thus, these studies indicate that improving leptin receptor signaling in the VMH improves glucose homeostasis and energy metabolism.

Although VMH neurons also express significant insulin receptor (Havranksova, Roth, and Brownstein, 1978) and VMH neurons respond to insulin (Davidowa and Plagemann, 2001; Spanswick et al., 2000), the physiological role of insulin on VMH neurons had not been addressed until very recently. Klockener et al. (Klockener et al., 2011) showed that insulin activates PI3K signaling in SF-1 neurons and reduces firing frequency in these cells by activating K_{ATP} channels. Deletion of the insulin receptor on VMH neurons restricted adiposity, leptin resistance and glucose intolerance associated with DIO. Intriguingly, deletion of the insulin receptor on VMH neurons increased the firing of anorexigenic POMC neurons in mice on a high fat diet. This result reveals that insulin-dependent PI3K signaling in VMH neurons influences POMC neuronal firing and contributes to the development of obesity, in contrast with the idea that the VMH is a ‘satiety center’. These results indicate that the function of the VMH cannot be assessed by simple lesion studies and that the regulation of appetite and energy metabolism in the VMH is more complex than originally appreciated. Although both leptin and insulin utilize the PI3K pathway in VMH neurons, leptin increases VMH cell firing whereas insulin inhibits VMH cell firing. This key difference presumably underlies the contrasting body weight phenotypes after insulin or leptin receptor deletion on VMH neurons.

Despite the well-described utility of SF-1 cre mice to probe questions around the function of VMH neurons, little is known about the biological function of SF-1 itself. Elmquist and colleagues recently deleted SF-1 from VMH neurons and discovered that this impaired energy
expenditure and increased susceptibility to DIO. Furthermore, these mice had reduced leptin receptor expression leading to leptin resistance (Kim et al., 2011). Thus, SF-1 is a critical transcription factor that programs the VMH to maintain energy homeostasis by regulating leptin receptor expression.

The Lateral Hypothalamus

Early studies showed that the lateral hypothalamus was the hypothalamic feeding center, because surgical lesions block feeding. Subsequent studies have identified two key neuronal populations in the lateral hypothalamus that regulate appetite; Orexin and melanin concentrating hormone (MCH) neurons. The orexin and MCH neurons are only found in the lateral hypothalamus and both stimulate feeding after icv injection (Rossi et al., 1997; Sakurai et al., 1998).

Orexin

The role of orexin on appetite has been questioned as the effects are relatively short (Edwards et al., 1999) and ob/ob and db/db mice have reduced prepro-orexin gene expression and peptide content (Stricker-Krongrad et al., 2002). Moreover, transgenic mice over-expressing orexin are resistant to diet-induced obesity and maintain insulin sensitivity by stimulating energy expenditure (Funato et al., 2009). Nevertheless, deletion of orexin causes hypophagia (Hara et al., 2001), fasting increases neuronal activation in orexin neurons and both fasting and hypoglycemia increase orexin mRNA (Diano et al., 2003; Sakurai et al., 1998). There is a strong interaction between orexin neurons and the melanocortin system, as orexin neurons synapse with AgRP and POMC neurons and NPY or AgRP neurons synapse with orexin neurons (Dube et al., 1999; Horvath et al., 1999). This circuit regulates the appetite-stimulating effects of orexin.

The regulation of orexin neurons by leptin is complex; for example, leptin inhibits neuronal firing of orexin neurons and blocks neuronal activation of orexin caused by fasting (Funato et al., 2009; Yamanaka et al., 2003). However, leptin stimulates preproorexin mRNA (Funato et al., 2009; Tritos et al., 2001; Yamanaka et al., 2003). The key to understanding this puzzle may lie in the leptin-sensitive neuronal circuitry in the lateral hypothalamus. Orexin neurons do not express leptin receptors and leptin injection does not increase pSTAT3 in orexin containing neurons (Leinninger et al., 2009b). However, leptin receptor neurons in the lateral hypothalamus express GABA (Leinninger et al., 2009b) and directly synapse with orexin neurons (Louis et al., 2010) and POMC neurons (Vong et al., 2011). Indeed, leptin may be a key hormone to explain the different effects of orexin on energy homeostasis. Although acute injection of orexin stimulates feeding, the long-term effect of orexin is to promote activity, energy expenditure and decrease feeding (Funato et al., 2009; Tritos et al., 2001; Yamanaka et al., 2003). The ability of leptin to stimulate orexin gene expression over prolonged periods may drive increased energy expenditure whereas the acute effects of orexin on food intake may require reduced leptin signaling, as seen during fasting (characterized by increased orexin neuronal activity and low leptin). More recent studies show a primary role of the orexin system in wakefulness (Funato et al., 2009; Yamanaka et al., 2003)
Ghrelin is also thought to activate orexin neurons to increase food intake in a direct and indirect manner via NPY neurons (Toshinai et al., 2003), however there is no evidence that orexin neurons express the ghrelin receptor and little evidence that the receptor is present in the lateral hypothalamus (Guan et al., 1997; Zigman et al., 2006). There is evidence that ghrelin stimulates orexin-dependent feeding through the dopaminergic reward pathways in the brain (Perello et al., 2010). Therefore, it seems likely that the orexin system in the lateral hypothalamus is important for ghrelin-induced food intake via indirect mechanisms involving the melanocortin system or the dopaminergic system. Future studies are required to elucidate the exact mechanism of action.

Orexin affects glucose homeostasis as icv infusion increases plasma glucose concentrations through an increase in hepatic glucose production that is blocked by hepatic sympathetic denervation. Interestingly, orexin stimulates hepatic glucose production in the same manner as NPY, suggesting that orexin neurons engage the melanocortin system to affect plasma glucose concentrations. Direct injection of orexin into the VMH stimulates glucose uptake in skeletal muscle through the sympathetic nervous system (Shiuchi et al., 2009) and overexpression of orexin restores glucose tolerance in DIO mice through an orexin-receptor 2 mediated action (Funato et al., 2009). These data collectively show that orexin plays important roles in peripheral glucose homeostasis through the sympathetic nervous system.

MCH

MCH neurons stimulate food intake, as MCH and MCH-1 receptor mice knockout mice are lean, hypophagic and more active than wild type controls (Marsh et al., 2002; Shimada et al., 1998). In accordance, MCH overexpressing mice are overweight and more susceptible to DIO (Ludwig et al., 2001). Classic obesity models such as ob/ob and db/db mice also show increased MCH mRNA in the lateral hypothalamus and this can be reversed by leptin treatment, suggesting MCH contributes to hyperphagia and weight gain (Qu et al., 1996; Tritos et al., 2001). Similar to the orexin system, α-MSH from POMC neurons in the melanocortin system inhibits the activity of MCH neurons. MCH can antagonize the anorectic effects of α-MSH via indirect mechanisms since MCH does not prevent α-MSH binding to the MC4R or MC3R (Ludwig et al., 1998; Tritos et al., 1998). Despite the ability of leptin to suppress MCH, these neurons do not contain leptin receptors (Leinninger et al., 2009a) and unlike orexin neurons, do not receive inputs from leptin-receptor containing neurons in the lateral hypothalamus (Louis et al., 2010). Thus, leptin receptor neurons, outside the lateral hypothalamus, must regulate the robust effect of leptin to suppress MCH expression. Leptin-induced activation of POMC neurons is the prime candidate for this mechanism. This still requires further proof.

The role of MCH in glucose homeostasis is poorly defined. Although MCH overexpression causes obesity and hyperglycemia (Ludwig et al., 2001), icv injection of MCH had no effect on plasma glucose levels in wild type, MCH knockout or MCH1R knockout mice (Yi et al., 2009). These results suggest that the hyperglycemia seen in MCH overexpressing mice may be due to the adiposity rather than overexpression of MCH. However, one recent paper showed that MCH neurons sense blood glucose levels and adjust their output to maintain a euglycemic state in the periphery (Kong et al., 2010). The
interaction between MCH neurons and peripheral metabolic hormones, such as leptin and insulin, in blood glucose control remains to be determined.

Recent studies also show that the lateral hypothalamus is also an important relay nucleus connecting basic homeostatic functions with higher cognitive function. The lateral hypothalamus integrates social, cognitive, rewarding and emotional aspects of palatable food, which can override the homeostatic appetite systems. Consistent with these ideas, the lateral hypothalamus neurons project to and receive inputs from, reward-associated brain regions, such as the ventral tegmental area and the nucleus accumbens (Fadel and Deutch, 2002; Leinninger et al., 2009a; Peyron et al., 1998).

These results described above highlight the importance of the lateral hypothalamus in appetite regulation and energy homeostasis. Neurons in lateral hypothalamus, both MCH and orexin, are directly wired into the ARC melanocortin system and dopaminergic reward system. Therefore, the idea that the lateral hypothalamus is the ‘feeding center’ may be misleading, as the orexigenic output may come from increased ARC AgRP neuronal activity, suppressed POMC neuronal activity and elevated dopamine reward pathways associated with palatable food intake. With the benefit of 60 years of research hindsight, the lateral hypothalamic ‘feeding center’ should incorporate the ARC melanocortin and the dopamine reward system.

Hypothalamic Synaptic Plasticity Regulates Food Intake

Synaptic plasticity is a term that describes changes in synaptic connections between two cells. This plasticity manifests in many different ways such as the quantal release of neurotransmitter at the synapse and the absolute number of synaptic contacts on a particular cell. There are two main types of identifiable synapses at the electron microscopic level: asymmetric excitatory glutamatergic synapses and symmetric inhibitory GABAergic synapses. Synaptic plasticity is a term classically associated with memory formation and hippocampal function but pioneering recent work shows that synaptic plasticity in hypothalamic neurons plays an important role in the regulation of appetite and the maintenance of energy balance.

The first evidence came from studies on leptin-deficient ob/ob mice. Pinto et al 2004 (Pinto et al., 2004), showed hyperphagic ob/ob mice had increased excitatory synapses and decreased inhibitory synapses on NPY neurons, whereas POMC neurons showed reduced excitatory synapses. This study shows that the synaptic organization of the melanocortin system in ob/ob mice dramatically favors NPY activation and subsequent hyperphagia. When leptin was administered systemically to ob/ob, synaptic input organization normalized to wild type levels within 6 hours, several hours before an observable effect on feeding. The discovery that leptin could modulate synaptic plasticity in the hypothalamus lead the same laboratory to investigate whether this is a general phenomenon for all metabolic hormones acting in the hypothalamus, or rather a leptin-specific effect on the melanocortin system. Accordingly, the anorexigenic effects of estrogen caused an increase in excitatory synaptic number on POMC neurons in a leptin-independent manner, as estrogen was still effective in ob/ob and db/db mice (Gao et al., 2007). Moreover, the orexigenic hormone ghrelin can also regulate synaptic plasticity on POMC neurons in the ARC nucleus. Ghrelin shifted the synaptic profile of POMC neurons in the opposite direction caused by leptin, for example.
ghrelin decreased inhibitory inputs on POMC neurons thereby reducing satiety drive through reduced activation of POMC neurons (Andrews et al., 2008; Pinto et al., 2004). This is a particularly intriguing observation as ghrelin does not act on POMC neurons and less than <8% of POMC neurons contain the ghrelin receptor (Willesen et al., 1999). The ghrelin-induced synaptic rearrangement on POMC neurons is due to GABAergic inhibitory inputs directly from AgRP neurons (Andrews et al., 2008). Corticosterone also produced synaptic rearrangement in AgRP and POMC neurons to favor increased food intake, i.e. increased inhibitory synapses on POMC and increased excitatory synapses on AgRP neurons (Gyengesi et al., 2010).

These studies clearly demonstrate that hormone-driven synaptic plasticity in the melanocortin system influences feeding behavior (Figure 7). However, many questions remain unanswered, for example do fuel metabolites, such as glucose and fatty acids, affect synaptic plasticity in a similar manner to hormones. Considering that glucose and fatty acids have direct effects on POMC and AgRP neuronal function and firing, and free access these neurons in the ARC, it is highly likely that fuel metabolites affect synaptic plasticity. Indeed, metabolic status also appears to mediate synaptic plasticity, perhaps offering the first clue that fuel metabolites, such as glucose and fatty acids, may regulate synaptic plasticity. Diet-induced obesity (DIO) reduces the total number of synapses on POMC neurons due to an increase in gliosis and glial ensheathment around POMC neurons (Horvath et al., 2010).

DIO also affects NPY synaptic organization by reducing the number of excitatory synapses. These data suggest that high fat diet has a profound influence on the synaptic input organization of POMC and NPY neurons (Horvath et al., 2010).

The reactive gliosis that occurs after high fat diet appears to form a barrier between these neurons and endocrine signals from the blood, implying hormonal signals cannot initiate the appropriate synaptic rearrangement. The lateral hypothalamic orexin neurons are instrumental to trigger arousal but are also important in food intake and energy metabolism. These neurons exhibit an unusual synaptic input organization characterized by a large number of excitatory synapses and minimal inhibitory inputs (Horvath and Gao, 2005). An overnight fast, which elevates ghrelin and inhibits leptin, promotes the formation of more excitatory synapses and synaptic currents onto orexin neurons. Leptin treatment during a fast or refeeding effectively blocked the synaptic rearrangement on orexin neurons. This study demonstrates that synaptic plasticity occurs in other regions of the hypothalamus, not only AgRP and POMC neurons. It further emphasizes the role of metabolic status on synaptic reorganization and energy metabolism.

While these studies indicate the importance of synaptic plasticity on hypothalamic feeding circuits there are still many unanswered questioned. Most importantly, in these studies described above, the authors are examining only axo-somatic synapses (axon terminal synapses with target neuronal cell bodies), however the vast majority of synaptic input occurs within the dendritic field. The technical difficulties currently preclude examining the synaptic input organization at the level of the dendrite, however because changes in feeding behavior parallel axo-somatic synaptic input, it is reasonable to expect axo-dendritic inputs do the same. It remains to be determined what intracellular signaling mechanisms mediate changes in synaptic input and how this occurs. However, chronic PI3K activation in POMC neurons increases inhibitory synaptic input (Plum et al., 2006). Finally, because synapses are protracting and retracting around neuronal cell bodies and dendrites, there is a large demand
Synaptic plasticity regulates the activity of the melanocortin system in the hypothalamus. POMC neurons constantly undergo changes in synaptic inputs. These synaptic inputs can either be from inhibitory GABAergic neurons or from excitatory glutamergic neurons. The ratio of inhibitory/excitatory synaptic inputs influences the activity of the POMC neurons and has subsequent important implications in the hypothalamic control of appetite and energy metabolism. For example, hyperphagic ob/ob mice have decreased excitatory and increased inhibitory synapses, which suppresses POMC neuronal activity and contributes to hyperphagia and obesity. Metabolic hormones, such as leptin and ghrelin, regulate this synaptic plasticity. For example, leptin increases the number of excitatory synapses and decreases the number inhibitory synapses on POMC neurons, thereby increasing POMC neuronal activity, α-MSH release and anorexia. Ghrelin increases the number of inhibitory synapses on POMC neurons, which decreases POMC neuronal activity and increases appetite. Synaptic plasticity also occurs in other neurons controlling energy metabolism, such as orexin neurons (see text).
for local energy production. Indeed, enhanced synaptic activity increases mitochondrial trafficking to the active area (Li et al., 2004) and mitochondrial biogenesis increases neuronal plasticity (Lopez-Lluch et al., 2008). Therefore, determining how cellular bioenergetics status affects synaptic plasticity will be the next important breakthrough in the field. Initial observations show that impaired mitochondrial biogenesis in POMC neurons reduces synaptic turnover (Andrews et al., 2008). In UCP2 knockout mice, ghrelin did not increase inhibitory synaptic inputs on POMC and this attenuated ghrelin-induced food intake relative control mice (Andrews et al., 2008).

Post-Translational Modifications

The melanocortin system is the most well described hypothalamic feeding circuit. Important recent advances show that the POMC-derived peptide MSH undergoes extensive post-translational modification before becoming the mature α-MSH peptide, which is responsible for activating the MC4R and inhibiting food intake. α-MSH generated from inappropriate POMC post-translational processing, such as α-MSH1-12, does not suppress food intake.

Prohormone convertase 1 (PC1) initially cleaves POMC into pro-ACTH and β-lipotrophin (βLPT) and then further cleaves pro-ACTH into ACTH1-39. βLPT undergoes further processing to form β-MSH and β-endorphin. Prohormone convertase 2 (PC2) cleaves ACTH1-39 into ACTH1-17 and then mature α-MSH is generated by the serial actions of carboxypeptidase (CPE), peptidyl α-amidating monoxygenase (PAM) and an unidentified n-acetyltransferase (NAT) respectively. CPE removes the carboxy-terminal basic amino-acids from ACTH1-17 to form α-MSH1-14, which is then amidated by PAM to produce desacetyl-α-MSH1-13. Mature acetyl-α-MSH is finally produced through the actions of NAT. Consequentially, generating the anorectic α-MSH peptide in the hypothalamus requires a complex set of post-translational steps catalyzed by numerous enzymes (Figure 8).

Therefore, it is highly possible that any defect in the post-translational processing of α-MSH, could decrease hypothalamic α-MSH and lead to hyperphagia and obesity. Indeed, hyperinsulinemia in obese fa/fa mice is associated with a CPE mutation (Naggert et al., 1995) and suppressed CPE activity (Fricker et al., 1996). Recent studies showed that deletion of FOXO1 in POMC neurons increases CPE expression, which resulted in elevated α-MSH in the hypothalamus and reduced food intake. Moreover, CPE expression was decreased in DIO and CPE overexpression in the hypothalamic arcuate nucleus also reduces food intake. These studies clearly show that CPE activity in the hypothalamic arcuate nucleus regulates α-MSH peptide levels and maintains normal energy homeostasis. New evidence shows that another carboxypeptidase (prolyl carboxypeptidase; PRCP) controls appetite by regulating the degradation of α-MSH. PRCP null mice are significantly leaner than controls on either a chow or high fat diet. PRCP degrades active α-MSH1-13 to inactive α-MSH1-12, which has no appetite-suppressing effect when injected centrally (Wallingford et al., 2009), and α-MSH1-12 does not activate action potential firing in MC4R neurons in the PVN. Furthermore, a human PRCP mutation is associated with the metabolic syndrome in males (McCarthy et al., 2003). AgRP also undergoes post-translational modifications that affect antagonistic activity at the
MC4R. PC1 cleaves unprocessed full length AgRP and generates an AgRP\textsubscript{83-132} peptide that has greater antagonistic activity at the MC4R. Moreover, PC1 null mice have 3.3-fold more unprocessed AgRP compared to controls (Creemers et al., 2006). PCs also play important roles in proNPY processing (Brakch et al., 1997). The ability of PC1 to regulate POMC, NPY and AgRP may have important implications for overall energy metabolism. Indeed, human obesity is associated with a mutation in the PC1 gene (Ramachandrappa and Farooqi, 2011) and PC activity is regulated by metabolic state, leptin and obesity. Future studies are required to examine post-translational mechanisms regulating energy metabolism in the hypothalamus, as targeting these processing steps may be useful to control obesity.

Figure 8. Post-translational modifications required to produce active acetyl \(\alpha\)-MSH in POMC neurons. Prohormone convertase 1 (PC1) initially cleaves POMC into pro-ACTH and \(\beta\)-lipotrophin (\(\beta\)LPT) and then further cleaves pro-ACTH into ACTH\textsubscript{1-39}. \(\beta\)LPT undergoes further processing to form \(\beta\)-MSH and \(\beta\)-endorphin. Prohormone convertase 2 (PC2) cleaves ACTH\textsubscript{1-39} into ACTH\textsubscript{1-17} and then mature \(\alpha\)-MSH is generated by the serial actions of carboxypeptidase (CPE), peptidyl \(\alpha\)-amidating monooxygenase (PAM) and an unidentified N-acetyltransferase (NAT) respectively. CPE removes the carboxy-terminal basic amino-acids from ACTH\textsubscript{1-17} to form \(\alpha\)-MSH\textsubscript{1-14}, which is then amidated by PAM to produce desacetyl-\(\alpha\)-MSH\textsubscript{1-13}. Mature acetyl-\(\alpha\)-MSH\textsubscript{1-13} is finally produced through the actions of NAT. \(\alpha\)-MSH\textsubscript{1-13} is degraded to the inactive \(\alpha\)-MSH\textsubscript{1-12} by the actions of prolyl carboxypeptidase.
Conclusion

The advent of novel molecular genetic techniques has dramatically aided our understanding of hypothalamic systems regulating appetite and metabolism. Indeed, many of the studies today are applying novel techniques to old questions, with a minor tweak. For example, Hetherington and Brobeck described the important roles for the lateral hypothalamus and the VMH in appetite and body weight. Today, scientists use sophisticated molecular genetics to tweak these early discoveries, for example deleting leptin or insulin receptor in these nuclei.

This chapter has systematically and in depth reviewed the current literature pertaining to how the hypothalamus regulates appetite and energy metabolism. Within the hypothalamus the ARC, VMH and LH are key nuclei regulating appetite and energy metabolism, however there is a general desire in the field to develop a more general understanding of how the brain regulates appetite and metabolism.

Indeed, new studies are beginning to focus on how the reward pathways regulate appetite or how higher cognitive centers influence feeding behavior at multiple levels. These approaches are likely to have significant impact on human health. Current technologies are limited to examining the effect of single gene deletions in specific neurons, and while this is an extremely useful tool to probe the role of single genes in neurons, it neglects to consider the integrative function of multiple genes in a neuronal system.

Future challenges will elucidate how neuronal networks in the hypothalamus integrate multiple hormonal and nutrient signaling molecules to regulate function. The next decade should begin to focus on translating these basic discoveries into treatment strategies.

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