Cell. Mol. Life Sci. (2011) 68:2569–2588
DOI 10.1007/s00018-011-0707-5

Cellular and Molecular Life Sciences

REVIEW

Melanocortin control of energy balance: evidence from rodent models

Bart C. De Jonghe · Matthew R. Hayes · Kendra K. Bence

Received: 3 March 2011 / Revised: 19 April 2011 / Accepted: 20 April 2011 / Published online: 8 May 2011

Abstract Regulation of energy balance is extremely complex, and involves multiple systems of hormones, neurotransmitters, receptors, and intracellular signals. As data have accumulated over the last two decades, the CNS melanocortin system is now identified as a prominent integrative network of energy balance controls in the mammalian brain. Here, we will review findings from rat and mouse models, which have provided an important framework in which to study melanocortin function. Perhaps most importantly, this review attempts for the first time to summarize recent advances in our understanding of the intracellular signaling pathways thought to mediate the action of melanocortin neurons and peptides in control of longterm energy balance. Special attention will be paid to the roles of MC4R/MC3R, as well as downstream neurotransmitters within forebrain and hindbrain structures that illustrate the distributed control of melanocortin signaling in energy balance. In addition, distinctions and controversy between rodent species will be discussed.

Keywords Melanocortin · POMC · ARC · NTS · Leptin · MC4R · MC3R · Obesity

Abbreviations

A^Y	Agouti yellow
AAV	Adeno-associated virus
ACTH	Adrenocorticotropic hormone
AgRP	Agouti-related protein
AMPK	Adenosine monophosphate protein kinase
BAT	Brown adipose tissue
BDNF	Brain-derived neurotrophic factor
CART	Cocaine- and amphetamine-regulated transcript
CCK	Cholecystokinin
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
DIO	Diet-induced obesity
DVC	Dorsal vagal complex
FI	Food intake
Fos-Li	Fos-like immunoreactivity
GABA	γ-Aminobutyric acid
GK	Glucokinase
GLP-1	Glucagon-like peptide 1
HFD	High-fat diet
IBAT	Intrascapular brown adipose tissue
ICV	Intracerebroventricular
IR	Insulin receptor
LHA	Lateral hypothalamic area
LPS	Lipopolysaccharide
MAPK/ERK	Mitogen-activated protein kinase (a.k.a. = extracellular signal-regulated kinase)
MCR	Melanocortin receptor
MC3R	Melanocortin 3 receptor
MC4R	Melanocortin 4 receptor
MSH	Melanocyte-stimulating hormone
MTII	Melanotan 2
NPY	Neuropeptide Y
NR	Not reported
NT-4	Neurotrophin-4
NTS	Nucleus tractus solitarius
NUCB2	NEFA/nucleobindin2

B. C. De Jonghe · K. K. Bence (✉)
Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
e-mail: kbence@vet.upenn.edu

M. R. Hayes
Department of Psychiatry, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

PI3 K	Phosphatidyl inositol 3-kinase
PBN	Parabrachial nucleus
PC	Subtilisin-related prohormone convertase
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
POMC	Proopiomelanocortin
PTP1B	Protein tyrosine phosphatase 1B
PVH	Paraventricular nucleus of the hypothalamus
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
RQ	Respiratory quotient
SHP2	SH2 domain-containing protein tyrosine phosphatase-2
SIM1	Single-minded gene 1
SNS	Sympathetic nervous system
SOCS3	Suppressor of cytokine signaling 3
SON	Supraoptic nucleus
STAT3	Signal transducer and activator of transcription 3
trkB	Tropomysosin-receptor kinase-B
WAT	White adipose tissue
Tg	Transgenic

Introduction

The prevalence of obesity within the US and several developing countries the world over [1–4] has catalyzed the need for a greater understanding of how physiological signals of food intake, thermogenesis, and energy expenditure converge within the brain to regulate body weight. A recurring theme that has emerged from decades of research using a multitude of human and animal models is that the melanocortin system appears to be an essential component of the overall regulation of energy balance [5–7]. The melanocortin "system" can be defined as a neural circuit comprised of cells expressing either pro-opiomelanocortin (POMC)-derived melanocortin receptor (MCR) agonists, or the melanocortin antagonist agouti-related peptide (AgRP), as well as the MCR-expressing cells that are targets of these neurons. Melanocortin peptides and associated receptors are found in almost every region of the body: from dense concentrations within specific sites of the brain [8], to peripheral tissues including the testes, duodenum, kidney, lungs, stomach, skin, and placenta [9, 10]. Such ubiquitous expression speaks to the diversity and wide array of physiological functions governed by the melanocortin system [6, 11, 12]. Foremost of these melanocortin-mediated functions is energy balance regulation as a result of MCR signaling within hypothalamic and hindbrain nuclei [13, 14].

Studies in rodents and humans have highlighted the importance of melanocortin signaling in the control of body weight [15]. Among the most compelling findings are those in mice showing that genetic knockout of POMC [16], or targeted destruction of POMC neurons [17], results in significant obesity and dysregulated energy balance. Similarly, overexpression of AgRP [18] leads to hyperphagia and obesity, while targeted destruction of AgRP neurons in adult mice [17, 19] leads to lowered body weight and anorexia. In humans, POMC mutations have also been observed in patients with severe early-onset obesity [20].

Many hormones have been implicated in the regulation of melanocortin signaling, including the adipocyte-secreted hormone leptin. With the discovery and identification of leptin in 1994 [21, 22], it quickly became clear that some of the most well-studied obese rodent models including the Zucker rat [23], as well as *ob/ob* and *db/db* mice [24], all developed excessive adiposity through a lack of leptin or absence of leptin receptor signaling. Either of these manifestations will lead to subsequent dysregulation of the melanocortin system. This breakthrough catapulted adipose tissue into the limelight as a source of secreted factors contributing to energy balcony control. In addition, it established the central nervous system (CNS), specifically the hypothalamus, a brain area previously well known to be involved in body weight regulation, as the staging ground for leptin's central effects [see [5] for review]. Since this time, mouse and rat models have been credited with providing the basis of our current knowledge on leptin's action within POMC and AgRP neurons, as well as the activation of many downstream signals. Arguably the most important secondary sites of action of the melanocortin system are melanocortin 3 receptor (MC3R)- and melanocortin 4 receptor (MC4R)-containing neurons within the arcuate nucleus of the hypothalamus (ARC) and paraventricular nucleus of the hypothalamus (PVH) [25, 26]. These receptors and the neurons which express them have been heavily studied in both the mouse and rat through complementary approaches. MC4R dysregulation has been studied in detail in rodents [27, 28] and humans [29] and is perhaps the most prevalent monogenic variant in the susceptibility of human obesity [7].

This review will attempt to highlight how advances in rodent models over the last several years have helped establish a model of melanocortin control of energy balance from an intracellular, neuroanatomical, and neurochemical perspective. The use of Cre-LoxP recombination technology in mice has allowed for tissue- or neuron-specific knockout, and/or re-constitution of specific genes involved in melanocortin signaling. These mouse models have been critical in establishing the importance of various receptors and signaling molecules in melanocortin control of energy balance. Studies of the melanocortin system in the rat have primarily involved pharmacological

stimulation/inhibition of melanocortin signaling pathways via direct intraparenchymal delivery of agonists/antagonists into specific nuclei of the brain. As will be discussed in greater detail in the balance of this review, the activation of specific MCR types within the hypothalamic and hindbrain nuclei (e.g., MC3/4R), as well as potential transcription factors and downstream projection sites of melanocortin receptor-expressing neurons, reveal a distributed network of interactive sites in many regions of the brain which may cooperate, or function independently, to ultimately regulate energy balance [14].

POMC and AgRP function in energy balance

Alpha-melanocyte-stimulating hormone (α-MSH) is a potent endogenous ligand for both MC3R and MC4R, the two most prominent melanocortin receptor types involved in body weight regulation, and has been shown under many paradigms to reduce food intake and/or increase energy expenditure in rodents [30]. Conversely, agouti-related protein (AgRP) serves as the main endogenous peptide directly antagonizing the action of α-MSH by competing for binding on MCRs and inhibiting receptor activation [31]. For the purposes of this review, we will focus primarily on the role of α-MSH and AgRP peptides and associated MC3R and MC4R binding in melanocortin action of energy balance.

Differential cleavage of POMC produces melanocortin peptides

The production of all melanocortin peptides occurs via cleavage of the POMC precursor in one or more steps [6, 9, 32]. Posttranslational cleavage of POMC is accomplished through the actions of two subtypes of the subtilisin-related proprotein convertase: PC1/3 and PC2 [33, 34]. Depending on the site of POMC expression, the peptides produced may be influenced by the presence or lack of convertase activity. For example, the principal end-product of POMC cleavage in corticotrophs of the anterior pituitary is adrenocorticotropic hormone ($ACTH^{1-39}$), which results after two successive cleavage steps via PC1/3 [32]. It is worth noting that in this region of the brain there appears to be no inherent PC2 activity [34], suggesting regional control of melanocortin production at the cleavage level. However, this notion may be more complex, as animals that genetically lack PC1/3 still manage to produce ACTH, suggesting a degree of plasticity under certain developmental conditions [35]. In the ARC, the nucleus tractus solitarius (NTS) of the hindbrain, and the melanotrophs of intermediate lobes of the pituitary, POMC processing continues beyond ACTH via PC2 and multiple additional enzymatic steps to produce two principally occurring melanocortin peptides: desacetyl-α-MSH and α-MSH. It is still unclear how the relative concentrations of specific melanocortins may complement or antagonize the downstream effects of one another, yet it is interesting to note that, within the ARC, desacetyl-α-MSH is present in larger concentrations than α-MSH, whereas α-MSH is found in greater levels in the NTS (see [6] for review). The relevance of this apparent disparity is unknown; however, it may have implications for the overall melanocortinergic tone in each of these nuclei as it relates to energy balance regulation.

AgRP cleavage and melanocortin receptor binding in energy balance

Murine AgRP is a 131 amino acid peptide displaying a high degree of homology to the 132 amino acid human AgRP (hAgRP) peptide [36]. The posttranslational carboxy-terminal fragment $AgRP_{83–132}$ of hAgRP has been used for over a decade as a potent antagonist of MCR activity [37]. What is not so straightforward is how the cleavage, posttranslational processing, and mechanism of AgRP action on MCRs may collectively occur and influence melanocortin control of energy balance. Indeed, AgRP in the circulation largely corresponds to fragmented forms of the original peptide [36, 38]. This processing has been shown to occur within AgRP-containing neurons of the hypothalamus primarily via PC1/3, although other PCs may also be involved [39].

Processing of AgRP prior to release from AgRP neurons seems to be critical to the proposed mechanism of AgRP in melanocortin receptor binding. Specifically, syndecan-3, a cell surface proteoglycan, has been postulated to act as a co-receptor for AgRP on MC4R neurons within the PVH via binding of the amino-terminal end of AgRP [40]. Mice lacking syndecan-3 are more sensitive to the effects of the MC3/4R nonselective melanocortin agonist melanotan 2 (MTII) [41] and show decreased endogenous levels of AgRP in the PVH [42], suggesting a decrease in AgRP tone localized to the PVH due to less efficient AgRP binding in the absence of syndecan-3. However, in light of the evidence of pre-synaptic AgRP cleavage prior to release, the physiological relevance of syndecan-3-mediated AgRP action at MCRs is controversial, as the endogenous AgRP fragment reaching the PVH would likely not possess the necessary amino-terminal recognition sequence necessary for syndecan-3 binding [see [39] for additional discussion]. This notion is also supported by the recent finding that $AgRP_{83–132}$ increases intake comparably in wild-type and syndecan-3 knockout mice [42]. Together, these results suggest that pre-synaptic posttranslational cleavage of AgRP is important for energy balance control.

Location of POMC and AgRP neurons

Within the ARC, two distinct populations of neurons synthesize either AgRP or POMC, and mediate opposing anabolic or catabolic effects on energy balance. Circulating hormones, such as leptin and insulin, can act upon these "first-order" neurons to decrease appetite and increase energy expenditure via simultaneous suppression of AgRP neurons and stimulation of POMC neurons [5]. Indeed, hypothalamic AgRP neurons increase in basal firing rate during fasting, likely through leptin-dependent mechanisms [43]. POMC neurons are also expressed in the NTS of the hindbrain [44], a nucleus that receives and integrates both vagal afferent satiation and blood born energy status signals, and issues output commands essential to energy balance control [45–48]. Direct administration of leptin in the hindbrain is sufficient to produce decreased food intake and increased energy expenditure, and leptin receptor signaling in the NTS is required for normal control of energy balance regulation [49–51]. The function of POMC neurons within the NTS may differ significantly from those in the ARC, although the literature contains only a handful of reports addressing this issue. For example, one report has shown that leptin does not induce the phosphorylation of signal transducer and activator of transcription 3 (STAT3) or Fos-like immunoreactivity (Fos-Li) in POMC NTS neurons, in contrast to robust activation in ARC POMC neurons [52]. However, another report suggests the opposite, that pSTAT3 is induced in a significant population of POMC NTS neurons following leptin treatment [53]. The disparate findings, both in mice, may involve the genetic background of the animals, as well as the feeding state when leptin treatment occurred [52, 53]. Notably, both NTS and ARC POMC mRNA have been shown to decrease following periods of food restriction [52]. More recent data in the rat show that NTS-specific expression of POMC ameliorates chronic diet-induced obesity (DIO) and metabolic defects, while identical treatment in the ARC produces only transient improvements [54]. In an obese rat model, recombinant adeno-associated viral POMC gene delivery (rAAV-POMC) into the NTS results in long-term sustainable reductions in food intake, body weight, and improved insulin sensitivity in contrast to rAAV-POMC delivery to the ARC, which results in only transient improvements in these parameters [55]. These differences presumably involve compensation by AgRP activity, which is restricted to expression in the ARC, and is not found in the hindbrain. Overall, these reports in mice and rats suggest divergent roles of hindbrain and forebrain POMC neurons on energy balance depending on location within the brain, highlighting both brainstem and hypothalamic POMC neurons as potential sites of energy balance regulation.

First-order melanocortin neurons in the ARC send projections to other areas of the brain to coordinately regulate energy balance. For example, α-MSH-containing neurons in the ARC activate downstream secondary neuronal populations within proximal nuclei of the hypothalamus, e.g., the lateral hypothalamic area (LHA) and PVH, as well as at distant sites such as the parabrachial nucleus (PBN), amygdala, and the NTS [5]. Notably, ARC-originating α-MSH projections have axons which terminate in proximity to gastric distension-responsive neurons in the NTS, suggesting coordination of melanocortin tone and satiation signals arising from the gastrointestinal tract [56]. As described in detail later in this review, ARC and PVH neurons express melanocortin receptors relevant to body weight regulation and serve as a branch point for activation of many central melanocortin-induced neurons.

Signaling pathways within POMC and AgRP neurons regulate energy balance

Leptin and insulin signaling in POMC and AgRP neurons

The generation and characterization of POMC-Cre and AgRP-Cre mice in 2004 has allowed investigators to utilize these genetic tools to probe the function of signaling components of the melanocortin system as it relates to energy balance [57, 58]. The POMC-Cre; $Lepr^{flox/flox}$ mice were the first mice published using this technology, providing a unique model in which to study the role of leptin signaling specifically in POMC neurons [58]. These mice are obese, highlighting the important homeostatic role for POMC neuron leptin signaling in control of body weight. However, the extent of obesity in these mice is less than might be predicted given the well-established prominent role of leptin in regulating neuropeptide expression and POMC neuron activity, and the importance of α-MSH on downstream targets that regulate energy balance. This study, as well as several follow-up studies, highlight both the important contribution of POMC- and AgRP-neuron leptin signaling to overall energy balance, and the need to consider possible melanocortin receptor-independent effects of leptin [58–63].

Two proteins have been identified as important negative regulators of leptin signaling: suppressor of cytokine signaling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B) (reviewed in [64]). As expected, deficiency of either SOCS3 or PTP1B in POMC neurons results in reduced body weight and adiposity (due to enhanced leptin sensitivity and increased energy expenditure) when mice are maintained on a high-fat diet (HFD) [65, 66]. STAT3 is activated downstream of leptin signaling, and is a critical

signaling molecule which promotes many of the physiological effects of leptin [67–69]. Deficiency of STAT3 in POMC neurons results in an increase in body weight and adiposity of female mice only, and a decrease in POMC gene transcription, suggesting that leptin-induced STAT3 signaling within POMC neurons is only one component mediating the physiological effects of leptin [70]. Surprisingly, however, mice expressing a constitutively active version of STAT3 within POMC neurons (STAT3-C^{POMC} mice) develop hyperphagia and obesity on a chow diet due to chronic STAT3-induced upregulation of SOCS3, leading to a feedback inhibition of leptin signaling and the development of leptin resistance [71]. Collectively, these studies highlight the physiological relevance of leptin signaling in POMC neurons.

In addition to leptin, signaling stemming from the pancreatic hormone insulin has been shown to reduce food intake and increase energy expenditure and thus play an important role in the central control of energy balance [72, 73]. Insulin receptors (IR) are expressed throughout the CNS, including in the hypothalamus and NTS of the medulla [74–76]. Mice with neuronal deficiency of insulin receptors (NIRKO mice) demonstrate mild diet-sensitive obesity, yet the precise neurons mediating the effects on energy balance are not entirely clear [77]. Somewhat surprisingly, deletion of IR in either POMC neurons or AgRP neurons does not have any significant effect on overall energy balance parameters [78]. It should be noted, however, that reconstitution of IR into POMC neurons of mice with hypothalamic IR-deficiency (L1 mice), does rescue energy expenditure and locomotor activity defects of L1 mice [79]. No rescue occurs if IR is put back into AgRP neurons of L1 mice, suggesting that restoring IR signaling specifically in POMC neurons may play a more important role in regulating energy balance than insulin signaling in AgRP neurons. To complicate matters, mice lacking both leptin receptors and IR in POMC cells are leaner than mice lacking POMC-leptin receptors alone [63]. These data suggest that IR signaling in POMC neurons normally promotes or maintains body weight, and that the anorectic effects of central insulin are mediated by non-POMC and non-AgRP neurons.

Despite the lack of (or minimal) effect of manipulating IR's in POMC and AgRP neurons, genetic disruption of particular isoforms of downstream phosphatidyl inositol 3-kinase (PI3 K) can affect energy balance. PI3 K is composed of a regulatory 85-kDa subunit (p85) and a 110-kDa catalytic subunit (p110) [80]. Mice with POMC-p85α deficiency display a sex-specific body mass phenotype; male POMC-p85$\alpha^{-/-}$ mice are normal weight when maintained on chow or high-fat diet, whereas female POMC-p85$\alpha^{-/-}$ mice have reduced body weight and adiposity on HFD [81]. Although there are some discrepancies between the reported phenotypes (see Table 1), overall it appears that disruption of the p110α or p110β subunit of PI3 K in POMC neurons results in increased body weight and adiposity [81, 82]. Deletion of p110α in AgRP neurons does not alter energy balance, but mice lacking p110β in AgRP neurons have decreased body weight and adiposity on either chow or high fat diet, due at least in part to decreased food intake [82]. It is worth noting that disrupting PI3 K signaling in arcuate neurons does seem to significantly affect glucose homeostasis and peripheral insulin sensitivity [63, 81], a topic that we will not cover in this review. Overall, these studies are consistent with previous reports which highlight the importance of POMC neuron PI3 K signaling to effects on feeding [83], yet also suggest that neuronal PI3 K signaling may be more important in control of glucose homeostasis than in long-term control of energy balance [84]. In addition, POMC-specific ablation of the phosphatase which dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate (PIP3), phosphatase and tensin homolog (PTEN), results in hyperphagia, increased adiposity, and increased body weight [85]. This is likely due, however, to POMC neuron hyperpolarization and a reduction in firing rate due to increased ATP-sensitive potassium (K_{ATP}) channel activity rather than an effect on insulin receptor signaling per se.

Since 2004, numerous studies have been published analyzing the metabolic role of various signaling molecules in POMC or AgRP neurons (see discussion above and Table 1). Most of these studies confirm the important contribution these neurons play in energy balance control. One note of caution in interpreting these studies comes from a recent report demonstrating that *Pomc* is widely expressed in immature embryonic hypothalamic neurons, many of which adopt a non-POMC cell fate in adult mice [86]. It is important to keep this caveat in mind when interpreting the energy balance phenotypes of mice which are lacking a gene of interest in any cell that has ever expressed POMC, compared with mice that may have a disruption of the gene in adult POMC neurons. To this end, the field seems to be moving toward more "conditional-inducible" transgenic mouse models to allow for better temporal resolution of specific gene functions.

Melanocortin receptors

Five MCR subtypes have been identified to date, each with some distinguishable (as well as overlapping) physiological characteristics. MC1R, MC2R and MC5R, do not appear to be essential for regulation of energy balance. Mutation in the recessive allele for MC1R in mice is not accompanied by a change in energy balance [6, 87]; similarly, MC2$R^{-/-}$

Table 1 A summary of genetic mouse models used in analysis of melanocortin signaling in the neuronal control of energy balance

Mutation/manipulation	Cell type	Body composition	Food intake	Energy expenditure/activity	Refs.
Lepr (Leptin receptor)	POMC	Increased BW and adiposity on chow and HFD; normal body length [58]; female KO mice accumulate more visceral adiposity [61]	Normal [58, 61]	Normal VO_2 in chow-fed males [58]; increased heat production in chow-fed males, decreased heat production in chow-fed females [61]; trend toward decreased locomotor activity in females only [61]	[58–61]
	AgRP	Increased BW and adiposity on chow diet	Normal	Normal RER, decreased locomotor activity, and reduced body temperature in chow fed males	[59]
	POMC + AgRP	Increased BW and adiposity (but normal lean mass) on chow diet compared to wild-type controls and compared to POMC-Lepr or AgRP-Lepr KOs; increased fat mass in females on HFD	Elevated food intake on chow during 4–7 weeks of age due to increased meal size (not number); no difference in FI of adult females on HFD	Reduced VO_2 and increased RER, decreased locomotor activity, and reduced body temperature in chow-fed males	[59]
Replace long form of the leptin receptor (ObRb) into POMC neurons of *db/db* mice	POMC	Reduced BW compared to *db/db* controls, but elevated compared to WT mice; length normal	Reduced food intake compared to *db/db* controls, but elevated compared to WT mice	Normal VO_2 and VCO_2 in males and females; increase in locomotor activity compared to *db/db* controls	[62]
IR (Insulin Receptor)	POMC	Normal BW and adiposity on chow and HFD; normal length	Normal	NR	[78]
	AgRP	Normal BW and adiposity on chow and HFD; normal length	Normal	NR	[78]
Replace IR selectively in POMC neurons of L1 mice (mice with hypothalamic deficiency of IR)	POMC	Decreased BW on chow compared to WT mice; normal fat mass	Modest increase in FI on chow compared to WT or L1 mice	Increased energy expenditure (VO_2) and locomotor activity compared to L1 mice	[79]
Replace IR selectively in AgRP neurons of L1 mice (mice with hypothalamic deficiency of IR)	AgRP	Decreased BW on chow compared to WT mice; normal fat mass	Normal	Similar EE profile as L1 mice	[79]
IRS2 (Insulin Receptor Substrate 2)	POMC	Normal BW and length	Normal	NR	[219]
Lepr and IR simultaneously	POMC	Deletion of both IR and Lepr in POMC cells reduces the elevated BW and adiposity seen in POMC-Lepr mice	Normal	Female double KOs show decreased VO_2 compared to WT controls (similar VO_2 to POMC-Lepr); female double KOs have normal locomotor activity, whereas POMC-Lepr females have decreased activity	[63]

Table 1 continued

Mutation/manipulation	Cell type	Body composition	Food intake	Energy expenditure/activity	Refs.
PTP1B (Protein tyrosine phosphatase 1B)	POMC	Reduced BW, adiposity, and length in males and females on HFD; normal lean mass	Normal	Increased VO_2 and body temperature compared to WT controls on HFD; normal activity	[66]
SHP2 (SH2 domain-containing protein tyrosine phosphatase-2)	POMC	Increased BW, adiposity, and length in males and females on chow or HFD; normal lean mass	Normal	Decreased VO_2 and activity compared to WT controls on HFD; normal body temperature	[66]
STAT3 (Signal transducer and activator of transcription 3)	POMC	Normal BW and adiposity in males on chow or HFD; increased BW and adiposity in females on chow but normal BW on HFD	Increased FI in females; normal FI in males	NR	[70]
STAT3-C (express constitutively active form of STAT3)	POMC	Increased BW, adiposity, and length on chow diet; normal BW, adiposity, and length on HFD	Increased FI on chow; normal FI on HFD	NR	[71]
	AgRP	Decreased BW and adiposity in males and females on chow diet or HFD	Normal on both diets	Increased VO_2 and locomotor activity compared to controls	[220]
SOCS3 (Suppressor of cytokine signaling-3)	POMC	BW normal on chow diet; decreased BW on HFD	Normal FI on chow or HFD	Increased VO_2 and RER compared to controls on HFD; normal activity	[65]
FoxO1 (forkhead box protein O1)	POMC	BW and adiposity decreased on chow diet or HFD; slight increase in relative lean mass	Decreased FI on chow	Normal energy expenditure and locomotor activity on chow diet; impaired calorie restriction-induced increase in locomotor activity on HFD	[221]
Gp130 (Glycoprotein 130)	POMC	Normal BW, adiposity and length on chow or HFD	Normal	Normal basal metabolic rate	[222]
PI3 K (Phosphatidyl inositol 3-kinase)					
p85-α on the background of global p85$\beta^{-/-}$	POMC	Normal BW, adiposity and length in males and females on chow or HFD	Normal	Normal VO_2 and RQ	[83]
p85-α	POMC	Normal BW in males and females on chow diet; normal BW and adiposity in males on HFD, but reduced BW and adiposity in females on HFD	NR	NR	[81]
p110-α	POMC	Increased BW in males and females on chow diet; normal length	Normal on chow	Normal activity; female KOs show decreased VO_2 and heat production with normal RER on chow diet	[81]

Table 1 continued

Mutation/manipulation	Cell type	Body composition	Food intake	Energy expenditure/activity	Refs.
p110-α	POMC	Normal BW, adiposity and length on chow diet; increased BW and adiposity on HFD	Normal on chow	Resting metabolic rate normal on chow diet	[82]
	AgRP	Normal BW and adiposity on chow diet or HFD	Normal on chow	Resting metabolic rate normal on chow diet	[82]
p110-β	POMC	Normal BW with increased adiposity and length on chow diet; increased BW and adiposity on HFD	Increased	Resting metabolic rate normal on chow diet	[82]
	AgRP	Decreased BW and adiposity on chow diet or HFD	Decreased on chow	Resting metabolic rate normal on chow diet	[82]
PTEN (phosphatase and tensin homolog)	POMC	Increased BW, adiposity and length in males on chow diet; transient increase in BW in males on HFD (weeks 9-15); normal BW and adiposity in females on chow diet; increased BW and adiposity in females on HFD	Increased in males on chow and females on HFD	Normal VO_2, RER, body temperature and locomotor activity in males on chow diet or in females on HFD.	[85]
mutKir6.2 (Tg overexpression of a mutant K_{ATP} channel subunit)	POMC	Normal BW in male Tg mice on chow diet	NR	NR	[205]
PDK1 (3-phosphoinositide-dependent protein kinase 1)	POMC	Transiently increased BW in males and females on chow or HFD (~6–9 weeks of age); decreased adiposity at 18 weeks of age	Increased FI at 8 weeks of age (males on chow diet), but normal FI at 10 weeks	NR	[223]
TSC1 (Tuberous sclerosis protein 1)	POMC	Increased BW and adiposity in male mice on chow diet	Increased food intake	NR	[224]
AMPKα2 (AMP-activated protein kinase, alpha 2 subunit)	POMC	Increased BW and adiposity in males on chow or HFD	No difference in daily ad libitum FI, but increased FI following fasting and re-feeding	Decreased resting metabolic rate (RMR) in males on chow diet	[225]
	AgRP	Decreased BW in males on chow diet after 14 weeks of age	Normal	Normal RMR	[225]
SIRT1 (NAD-dependent deacetylase sirtuin-1)	POMC	Normal BW, adiposity and length in males and females on chow diet; increased BW and adiposity on HFD, normal lean mass on HFD	Normal	Decreased VO_2 consumption, CO_2 production and heat production in females on HFD	[226]

or MC5R$^{-/-}$ mice do not exhibit obesity but may have defects in gluconeogenesis, adrenal atrophy, hypoglycemia, and exocrine functioning [88–90]. The most relevant and well-studied melanocortin receptors in the physiology of energy balance are the MC4R and MC3R, and the roles of these two receptor subtypes will be described in detail.

Effects of melanocortin MC4R and MC3R on energy balance

MC4R is critical to energy balance

The MC4R, a 7-transmembrane G-protein coupled receptor that shares structural similarity to the MC3R [91], is expressed on the surface of neurons throughout the brain and in high proportion within the hypothalamus, hippocampus, and hindbrain [31]. MC4R can be directly activated via ACTH or α-MSH, or inhibited by AgRP, via release from terminals of POMC or AgRP/NPY axonal projections from the ARC [31]. MC4R$^{-/-}$ mice show marked hyperphagia, hyperinsulinemia, hyperleptinemia, and obesity [28]. Selective MC4R re-expression in the PVH and amygdala can significantly blunt obesity development and completely block the hyperphagia otherwise seen in globally-deficient mice [92]. However, this tissue-specific MC4R re-expression does not affect the reduced energy expenditure phenotype of these animals, suggesting that at least in the PVH and amygdala, MC4R pathways regulate feeding and energy input, rather than output. Interestingly in the rat, AAV-mediated knockdown of PVH MC4R stimulates hyperphagia and DIO [93].

Given the wide distribution of the MC4R population within the CNS, identification of the relevant MC4R-expressing nuclei that regulate food intake has proven challenging. It is interesting to note that MTII induces neuronal activation, measured by Fos-like immunoreactivity (Fos-Li), in the PVH and area postrema (AP) of wild-type mice, while in MC4R$^{-/-}$ mice PVH Fos-Li remains intact, but AP expression is absent [94]. Other reports have also highlighted a role for MC4R signaling in food intake control using targeted parenchymal delivery of MTII in non-PVH nuclei [95–97]. Collectively, these data point to a distributed control of MC4R signaling within and between the forebrain and hindbrain in determining the obese phenotype of MC4R$^{-/-}$ mice.

Acute inhibition or activation of MC4R alters feeding and energy balance

In rat and mouse models, the use of selective or non-selective MC3R/MC4R agonists and antagonists has greatly contributed to the understanding of how MC4R neurons in the CNS regulate food intake. When administered intracerebroventricularly (ICV) in rats, selective blockade of MC4R increases both food intake and body weight [98–100]. Conversely, MC4R agonists given ICV reduce food intake in chow maintained [101] and DIO rats [102], suggesting that MC4R pathways are essential to the regulation of food intake.

The reduction of food intake observed in normal rodent models following administration of MTII is markedly attenuated [94] or blocked [103] in MC4R$^{-/-}$ mice. Similarly, double MC3R/MC4R$^{-/-}$ knockouts, as expected, do not reduce food intake following ICV MTII [94]. The failure of MTII to evoke a feeding response in MC4R$^{-/-}$ mice is due in large part to blockade of endogenous AgRP and leptin signaling, as these animals do not increase food intake following exogenous AgRP treatment and become resistant to leptin-induced anorexia when grossly obese [103]. Together, these data suggest that MC4Rs are likely the predominant MCR mediating the intake inhibitory effects of endogenous melanocortins and thus are the major CNS MCR responsible for mediating melanocortin's effects on energy balance.

An important recent publication illustrates that the stimulation of AgRP neurons involves a melanocortin receptor-independent mechanism to increase food intake, whereas POMC neuron activation requires intact melanocortin receptors to reduce food intake [104]. Feeding stimulatory effects of AgRP neuron activation is instead likely through GABA release/GABA-ergic signaling [105, 106]. These findings are not necessarily in contrast to results using ICV AgRP injections cited above in MC4R$^{-/-}$ mice, instead they demonstrate the significant distinction between methodologies of delivering a pharmacological compound (e.g., AgRP) versus the direct and indirect actions of AgRP neuronal excitation, the latter presumably involving a multitude of mechanisms not necessarily entirely due to release of AgRP.

Positive energy balance and increased adiposity can of course stem not only from hyperphagia but also decreased energy expenditure. In addition to inducing reductions in intake, MTII can increase core temperature and heart rate when administered into the forebrain or hindbrain [51, 107]. Interestingly, ventricular MTII does not appear to alter spontaneous activity in mice [108] or rats [51, 107]. Similarly, MC4R$^{-/-}$ mice do not show an alteration in physical activity when placed on HFD, whereas wild-type mice do [109]. On the other hand, specific re-constitution of MC4R within cholinergic neurons, likely through action of preganglionic sympathetic neurons, has recently been shown to increase energy expenditure, but not affect food intake [110]. It is also worth noting that specific allelic mutations of MC4R in humans can be associated with decreased energy expenditure as a likely cause of obesity in these individuals [111].

The melanocortin system mediates energy expenditure through activation and regulation of the sympathetic nervous system (SNS). Classical studies in rats with VMH lesions show profound obesity and large reductions in sympathetic activity, supporting a role for the VMH in energy balance though SNS action [112]. Parenchymal injection of leptin into the VMH, but not LHA, increases glucose uptake in SNS-target tissues including skeletal muscle, heart, and brown adipose tissue (BAT), but not in white adipose tissue (WAT) [113]. Leptin-stimulated glucose uptake in BAT is inhibited via VMH injection of SHU9119 and, conversely, MTII injections into either VMH or PVH stimulate glucose uptake in BAT [114]. If BAT is surgically sympathetically denervated, the aforementioned effects of leptin are completely blocked, suggesting that increased BAT uptake occurs via leptin-induced increases in sympathetic activation [113, 115]. These data show that leptinergic and melanocortinergic pathways are potent inducers of BAT activity via SNS activation. In the Siberian hamster, MTII increases lipid mobilization from WAT and increases "sympathetic drive" in BAT as evidenced by increased intrascapular BAT (IBAT) temperature and norepinephrine turnover [116]. Furthermore, intraPVH injections of MTII robustly increase IBAT temperature. It is likely that this effect is through MC4R receptor activation, particularly within the PVN, where a high density of SNS outflow projections to IBAT originates [117]. Taken together, these findings illustrate hypothalamic MC4R involvement in energy expenditure via the SNS regulation of BAT function.

MC4R activation stimulates anorexic signals within distinct nuclei and brain regions

CRH in the PVH

MC4R activation in the PVH can exert inhibitory actions on food intake through the stimulation of downstream effectors. Corticotropin-releasing hormone (CRH) is one such signal that reduces food intake when exogenously administered in rats [118]. Antagonism of the CRH receptor CRH-1 partially reduces MTII-induced reduction of food intake potentially through action in a small subset of PVH neurons that coexpress CRH and MC4R [119]. The anorectic action of CRH must be downstream of MC4R activation as CRH-induced reductions in food intake remain potent in obese $MC4R^{-/-}$ mice [103]. Together, these data show a role for CRH downstream of MC4R activation, likely within the PVH, to reduce food intake.

BDNF in the VMH, DVC, and Hippocampus

Classically examined as a contributor to neurodevelopment, brain-derived neurotrophic factor (BDNF) is thought to regulate energy balance as a downstream effector of MC4R [120]. Acute injections of BDNF can ameliorate hyperphagia and/or obesity in many animal models, including BDNF heterozygous mice, [121], agouti yellow (A^y), and *db/db* mice [120, 122], as well as in rats [123–125]. BDNF null mice die perinatally [126]; however, selective deletion of BDNF within the VMH and DMH results in hyperphagia and obesity, but not alterations in energy expenditure [127]. Similarly, knockout of the long form of the BDNF receptor, tropomysosin-receptor kinase-B (trkB), results in hyperhphagia and obesity when mice are maintained on a moderate-fat diet [120].

The control of intake by BDNF is distributed to multiple CNS locations, involving both forebrain and hindbrain action. BDNF expression in the VMH is reduced following food restriction in wild-type mice, increased after ICV administration of MTII, and less expressed in $MC4R^{-/-}$ mice. [120]. Evidence for BDNF involvement in hindbrain control of intake comes from a set of studies showing that BDNF and MC4R antagonist co-administration into the 4th ventricle of the rat attenuates increases in food intake due to MC4R antagonism alone [128]. Activation of the trkB receptor via BDNF, trkB agonist antibody, or neurotrophin-4 (NT-4), all suppress food intake and ameliorate obesity [129, 130]. Furthermore, hindbrain MC4R stimulation requires functional kinase activity of trkB to reduce intake [128]. Consistent with these data, it is likely that peripheral leptin-induced BDNF expression in the dorsal vagal complex (DVC) of the hindbrain [131] may be mediated by MC4R activation in the hindbrain**.** Additional BDNF populations, such as those within the hippocampus [132], may also play a role in feeding, specifically in the mediation of DIO [133]. As a whole, these data suggest multiple regulatory sites of BDNF/trkB regulation of MC4R-induced alterations in food intake. Although it remains unknown, it would be interesting to examine CNS distribution of melanocortin signaling in BDNF- or trkB-deficient rodent models, and whether or not the feeding effects of MC4R agonists or antagonists would remain intact in $BDNF^{+/-}$ or trkB knockout mice.

Sim1 and Oxytocin in PVH

Single-minded homolog 1 (Sim1) encodes a transcription factor critical to the proper neural development of the PVH, supraoptic nucleus (SON) [134], and potentially the LHA and amygdala [135]. Transgenic Sim1 overexpression in mice induces DIO resistance, but does not influence energy expenditure [136]. While the relationship between Sim1 action and MC4R is not well defined, several emerging reports clearly show that MC4R function in energy balance is impacted by manipulation of Sim1

expression. The obese A^y mouse, a genetic model for impaired melanocortin signaling [137], shows attenuated hyperphagia and obesity with overexpression of a Sim1 transgene [136]. These results suggest that Sim1 function may be involved in the rescue of melanocortin signaling efficiency in these animals. Interestingly, populations of Sim1 PVH neurons may also co-express MC4R, which is intriguing given that restoration of MC4R expression specifically within the PVH in obese MC4R-null mice largely prevents weight gain [92]. From both an anatomical and behavioral perspective, Sim1/MC4R function within specific PVH neurons may be essential for maintaining energy balance.

Similar to BDNF knockout mice discussed above, mice with homozygous germ line null mutations for Sim1 do not survive long after birth, likely due to deficient neural development. Heterozygous mice (haplotype $Sim1^{+/-}$), however, are viable and exhibit many deficits relating to energy balance, such as hyperphagia, hyperinsulinemia, hyperleptinemia, and obesity [138]. $Sim1^{+/-}$ mice show resistance to MTII-induced reductions in food intake and PVH Fos-Li, but normal MTII-induced increases in energy expenditure compared to wild-type mice [139].

Haploinsufficiency of Sim1 likely causes hyperphagia and obesity via downstream effects inhibiting oxytocin [140–142]. The selective MC4R-agonist cyclo(β-Ala-His-D-Phe-Arg-Trp-Glu)-NH_2 activates PVH oxytocin neurons in wild-type mice, while $Sim1^{+/-}$ mice are hypersensitive to the feeding inhibitory effects of oxytocin antagonism [143]. Postnatal Sim1-deficient mice, where Sim1 has been conditionally deleted shortly after birth, survive and show decreased hypothalamic oxytocin and MC4R mRNA in the PVH [140]. In summary, PVH Sim1 and oxytocin signaling may act in conjunction with, or downstream of, MC4R activation in the regulation of energy balance, although important components of the mechanism remain elusive, in particular how Sim1 may selectively affect food intake, but not energy expenditure.

NUCB2/Nesfatin-1 in the hypothalamus and NTS

Within the past few years, NEFA/nucleobindin-2 (NUCB2) has been identified as a precursor and potential mediator of melanocortin signaling in controlling food intake and body weight via cleavage into the active peptide fragment nesfatin-1 [144]. NUCB2 is expressed in multiple hypothalamic nuclei (PVH, ARC, LHA, SON) as well as the NTS [144], and peripheral tissues [145]. ICV injection of either NUCB2 or nesfatin-1 reduces food intake in Wistar [144], Sprague–Dawley [146], and lean or obese Zucker rats [144], and peripheral injection of nesfatin-1 is effective in dose-dependently reducing food intake in *db/db* and DIO mice [147]. Thus, in multiple animal models, including those of leptin resistance/absence, NUCB2/Nesfatin-1 is a potent mediator of food intake.

Interestingly, fasting appears to decrease both NUCB2 and nesfatin-1 levels only within the PVH, while α-MSH injection increases PVH expression of NUCB2 [144]. Fos-Li is robustly increased in PVH nesfatin-1 neurons subsequent to a 2-h refeeding following a 48-h fast [148], suggesting that these neurons are responsive to food deprivation. Along these lines, SHU9119, a selective MC3/4R antagonist, blocks the anorexic effect of nesfatin-1 [144] as well as oxytocin [149]. Similarly, oxytocin receptor antagonism via ornithine vasotocin blocks α-MSH- and oxytocin-induced anorexia in Sprague–Dawley rats [150] and nesfatin-1-induced anorexia in the Zucker rat [149]. Recent data also point to an inhibitory effect of nesfatin-1 in orexigenic neuropeptide Y (NPY) neurons of the ARC, which could also be a mechanism mediating food intake reductions induced by nesfatin-1 [151]. However, this relationship is unclear, as POMC and cocaine- and amphetamine-regulated transcript (CART) neurons of the NTS are activated at peripheral doses of nesfatin-1 that reduce food intake in mice [147], while in rats nesfatin-1 injection does not affect ARC/PVH expression of POMC, NPY, AgRP or CRH [144]. Taken together, these data suggest that the role of nesfatin-1 in food intake regulation involves MCR activation and/or participation of proposed downstream mediators of MCR signaling, such as oxytocin.

MCH and Orexin in the LHA

Melanin-concentrating hormone (MCH), a peptide found predominantly in the LHA, shows reduced expression after feeding and stimulates food intake when administered ICV [152] or parenchymally into the PVH [153], while MCH-1 receptor blockade reduces food intake [154]. MCH is capable of antagonizing the feeding effects of α-MSH, but not α-MSH binding to MC4R [153, 155], suggesting a downstream role in the MC4R-induced anorectic effect. Indeed, MCH expression is reduced by specific MC4R inhibition [156]. $MCH^{-/-}$ mice are hypophagic and lean, with reduced plasma leptin levels and ARC POMC expression, suggesting impaired melanocortin signaling [157]. Furthermore, A^y mice show increased MCH expression, which may mediate hyperphagia downstream of melanocortin signaling defects in these mice [158, 159].

Orexin in the LHA represents a potential downstream signal of melanocortin receptor activation that may show significant divergence in energy balance function between the mouse and the rat. Orexin expression in the LHA is increased in $POMC^{-/-}$ mice, and ICV injection of α-MSH reduces expression to WT levels [160], although administration of orexin is unable to significantly alter food intake

in both WT and MC4R$^{-/-}$ mice [103]. Furthermore, models of orexin deficiency in mice have produced contrasting results, showing that knockout of orexin precursor prepro-orexin and destruction of orexin-containing neurons both induce slight hypophagia [161, 162]. On the other hand, HFD induces obesity in animals lacking orexin neurons when mice are on a mixed genetic background [161]. Thus, the role of orexin in energy balance in mice is not straightforward, although melanocortinergic involvement is likely.

In the rat, directed chemical destruction of ARC NPY or POMC neurons results in hyperphagia and obesity and induces a downregulation of both hypothalamic MCH and orexin mRNA expression [163]. AgRP induces Fos-Li in orexin, but not MCH neurons of the LHA [164], while conversely, ICV injection of the MC3/4R antagonist, SHU9119, or AgRP increases MCH mRNA, but does not affect orexin mRNA [159]. Together, these reports show responsiveness of MCH and orexin to inhibition or stimulation by melanocortinergic signals, although the precise mechanisms remain unclear.

Brainstem MC4R can integrate peripheral signals of satiation and energy balance independently and via crosstalk with forebrain signals

The brainstem has long been recognized as an integrator of both short-term satiety signals and long-term signals of energy balance. In this regard, it has been shown that exogenous cholecystokinin (CCK)-induced reductions in food intake are blunted in MC4R$^{-/-}$ mice, and SHU-9119 administered into the PVH attenuates CCK-induced anorexia in the rat [165], although the physiological role of CCK and gut peptide response mediating in MC4R effects on intake are controversial [166–168].

Evidence using a decerebrate rat model suggests that brainstem activation of MCRs is sufficient to induce increases in core and BAT temperature, and in heart rate [169], although in an intact animal, it is likely that the these effects are mediated by both local activation within the brainstem and hypothalamus, as well as descending or reciprocal connections between the two areas [170]. Several brainstem populations, such as the NTS, PBN and raphe pallidus, contain sympathetic pre-motor neurons [171], and injection of MTII activates these areas, stimulating thermogenic mechanisms and energy expenditure [51, 169, 172]. Interestingly, forebrain or hindbrain injection of MTII comparably elevates UCP-1 in BAT, of which the effects of the latter are blocked following surgical denervation of BAT [172].

In addition to integration of peripheral signals, the NTS receives direct descending α-MSH containing projections from forebrain POMC neurons in the ARC [173], as well as MC4R expressing projections from the PVH [165]. Downstream effectors of MCR activation, namely oxytocin and CRH, induce Fos-Li in the NTS and brainstem [174, 175]. Similarly, subpopulations of both PVH oxytocin and CRH cells [176, 177], as well as LHA orexin and MCH-containing neurons, all project to the NTS [178, 179]. Together, these results suggest that local actions of melanocortin signals within the hypothalamus and hindbrain, as well as projections between the two, may cooperatively and/or independently regulate food intake and energy balance.

MC3R

MC3R is essential for energy balance

Like MC4R, the expression of MC3R is present throughout the brain, and is highly pronounced in regions of the forebrain and hindbrain involved in the control of food intake and energy balance, such as the ARC and NTS [180]. Endogenous melanocortins, such as α-MSH, potently bind to both MC3R and MC4R receptors [181], which initially suggested potentially redundant or overlapping roles of MC3R and MC4R. However, peripheral administration of a selective MC3R agonist stimulates food intake [182]. Furthermore, unlike MC4R$^{-/-}$ mice, MC3R$^{-/-}$ mice are hypophagic on chow [27], and do not exhibit hyperphagia on HFD despite the development of mild obesity on HFD [27, 183]. Decreased voluntary energy expenditure and spontaneous locomotor activity is also apparent in MC3R$^{-/-}$ mice, along with increased adiposity, reduced lean mass, and elevated respiratory quotient when switched to high-fat diet [27, 183] suggesting that the positive energy balance phenotype is likely due to decreased energy expenditure and lipid oxidation. Double knockouts for both MC4R and MC3R exhibit higher weight gain than either deletion alone [27], also supporting a role for independent effects of both receptor types in energy balance regulation.

MC3R activation may regulate melanocortin signaling through auto-inhibition of ARC neurons

The co-expression of MC3R on POMC and AgRP neurons within the ARC is particularly important. MC3R mRNA is expressed in about half of POMC [184, 185] and AgRP [185] cells located in the rostral section of ARC, while no such expression is found for MC4R. The role of these MC3Rs is thought to be auto-inhibitory, serving as a messenger in a feedback loop within and between ARC neurons and projection sites, such as the PVN, to maintain melanocortinergic tone and regulate POMC activity. The specific mechanism for the auto-inhibition may be through

inhibitory input via γ-Aminobutyric acid (GABA)-ergic terminals onto ARC NPY/AgRP neurons expressing MC3R and/or direct inhibition via MC3R activation on POMC neurons themselves [186].

MC3R as a mediator of circadian and entrainment patterns of food intake

MC3R also appears to have a regulatory role in the entrainment of food intake, under both restrictive and ad libitum feeding conditions ([187, 188], and for review, see [189]). Restrictive, scheduled feeding induces anticipatory behaviors characteristic of food expectation. Unlike wild-type mice, $MC3R^{-/-}$ mice do not show increased voluntary or spontaneous activity prior to scheduled meal presentation and exhibit abnormal oscillation patterns of rhythmically expressed clock genes such as Bmal1, Npas2 and Per2 when ad libitum fed [188]. These alterations in circadian patterns related to food intake also appear to extend to metabolic pathways, as hyperinsulinemia, glucose intolerance, and lipid metabolism impairments in $MC3R^{-/-}$ may be caused in part by defects in rhythmic expression in a number of transcription factors involved in liver function and metabolism of nutrients [187].

Peripheral signals from the gut activate central melanocortin signaling

Melanocortin signaling mediates the action of gastrointestinal signals and peptides

There is a significant body of literature showing communication between satiation signals arising from the gut and the central melanocortin system. Gastric nutrient infusion, for example, produces Fos-Li in α-MSH immunoreactive neurons within the NTS, suggesting that melanocortin signaling mediates nutrient signals from within the gut during digestion [173]. Furthermore, CCK-induced suppression of food intake occurs following vagal afferent activation and subsequent CNS processing that begins with hindbrain nuclei, including neurons involved in leptin and MC4R signaling [166, 190, 191]. Within the NTS, POMC neurons depolarize and increase firing rate and show increased Fos-Li in response to exogenous CCK treatment [192]. More recent data suggest that activated MAPK signaling within NTS POMC neurons plays a significant role in CCK-induced satiation [193]. The actions of CCK are not limited to the hindbrain. Intriguingly, forebrain melanocortin neurons in the PVH have also been shown to mediate the effects of CCK, in addition to hindbrain action [165, 194]. Similarly, projections containing downstream effectors of melanocortin receptor activation, such as oxytocin, appear to provide a neurochemical link between hypothalamic and NTS action of CCK [176, 195]. Interestingly, chronic decerebrate rats reduce food intake following CCK administration in the absence of brainstem–forebrain neural communication [196], suggesting that hindbrain action may be sufficient to mediate the reduced intake effects of CCK.

In addition to CCK, the intestinal hormone glucagon-like peptide-1 (GLP-1) may act through central mechanisms to reduce food intake [197] via melanocortin neurons. In the rat, GLP-1 injection attenuated fasting-induced decreases in POMC/CART expression, and conversely, reduced increases in AgRP/NPY expression [198]. Worth noting, however, is evidence of a divergence between mice and rats in leptin-induced pSTAT3 expression in NTS neurons containing proglucagon, the precursor for GLP-1. Specifically, in the rat, GLP-1 NTS neurons show no pSTAT3 expression following leptin treatment, while in mice, 100% of GLP-1-positive NTS neurons show expression of pSTAT3 [199]. This apparent conundrum requires further investigation, but warrants caution in similar interpretations of GLP-1/melanocortin signaling communication in each species.

Glucose sensing by central POMC neurons

The melanocortin system is involved in the meditation of nutrient signaling through specialized ARC, VMH, or NTS neurons which sense glucose, responding by excitation or inhibition when exposed to high or low circulating concentrations of the nutrient [200–202]. One of the main glucose sensors within neurons, the hexokinase glucokinase (GK), is found within VMH POMC neurons, and may control glucose sensing via K_{ATP} channels in glucose-excitatory POMC neurons [203–205]. Glucose sensing is impaired in DIO rats, as evidenced by reduced VMH GK expression [203], or defective uncoupling protein 2-regulation of glucose-induced ATP production in POMC neurons in mice [205], suggesting POMC glucose sensing pathways are critical to energy balance. α-MSH may also excite non-POMC-containing ARC neurons [206], and this pathway may require functional glucose transporter 2 expression in cells of the LHA and DVC that then project back to POMC neurons [207]. Overall, a role for POMC neurons in glucose sensing in the control of glucose homeostasis appears likely, and may involve multiple sites of action and cell type-specific sensor mechanisms.

Lessons from both the rat and mouse in melanocortin signaling of energy balance

As discussed in detail above, both rat and mouse models have been extensively studied in the melanocortin

system-mediated control of energy balance. While the overarching findings are often consistent, there are clearly some reported differences between the species. An all inclusive list of these differences would not be justified, as it is very possible that some of these differences are due in part to varying experimental techniques and pharmaceutical and genetic manipulations themselves. Instead, here we highlight a few interesting comparative and differential findings as they relate to melanocortin-mediated effects on energy balance between the rat and mouse. At least two important points can be made with regard to these comparisons: (1) the question of which species (rat or mouse) represents the appropriate model to understand the normal physiology and pathophysiology of human diseases is not straightforward, and will always depend on the physiological system under investigation; and (2) caution should be taken when making generalizations to human physiology from individual studies using the mouse or rat regarding the role of the melanocortin system in energy balance regulation.

Perhaps no region of the CNS has gained more attention in POMC-mediated effects on energy balance than the hypothalamus. Critical among the hypothalamic nuclei mediating the intake inhibitory and energetic responses to MC3/4R stimulation is the neuronal communication between the ARC and PVH as discussed above. Stimulation of MC4R in the PVH of the rat [208] or hamster [117] engages thermoregulatory responses that include increased core temperature, heart rate and locomotor activity, as well as reduced food intake and body weight. In contrast, MC4R re-expression in the PVH of MC4R knockout mice does not result in changes in energy expenditure [92], only amelioration of the hyperphagia of these mice. In the mouse, it appears that the energy intake and energy expenditure effects of the central melanocortin system are controlled by anatomically distinct portions of the system; namely, that food intake is regulated principally by forebrain MC4R-expressing nuclei, and hindbrain MC4R-expressing nuclei are regulating energy expenditure responses [92]. On the other hand, several studies in rats have shown clear food intake regulation by MC4R stimulation and/or blockade in the caudal brainstem [96, 208–210]. Specifically, Wan et al. [211] have shown that MC4R signaling in the NTS leads mainly to presynaptic modulation of glutamatergic synaptic transmission arising from the GI tract and suggests that melanocortinergic-induced decrease of food intake in the caudal brainstem may occur via enhancement of vagal afferent satiation signals from the gastrointestinal tract. Although there may be some differences between rat and mouse models, it is likely that stimulation of MCRs in a variety of central sites, including those within the hypothalamus and caudal brainstem, reduces food intake and increases energy expenditure.

The essential role of the CNS melanocortin system in energy balance regulation is attractive for the future development of pharmaceuticals aimed at treating not only obesity but other disease states affecting ingestive behavior. The pathogenesis of cachexia and malnutrition (specifically decreased food intake) caused by chronic disease or infection appears to involve the melanocortin system [212–215]. Various rodent models of cachexia exist in the rat and mouse, and include but are not limited to lipopolysaccharide (LPS) treatment, tumor implantation, and uremia-associated or cardiac injury-induced cachexia. The MC4R is reportedly involved in cachexia-induced suppression of food intake and alterations in basal metabolism, as MC4R null mice or wild-type mice treated with AgRP maintain their food intake and body weight when exposed to these various cachexia-induced paradigms (see [216, 217] for review). At least for cachexia it would appear that the rat and mouse models are consistent with a role of MC4R participation in regulating the disease-induced anorexia. Similar to the aforementioned findings with MC4R null mice, tumor-induced cachexia was prevented by treatment with the MC3/4R antagonist SHU-9119 in the rat [218]. Likewise, in both murine and rat models of heart failure, genetic and pharmacologic blockade of melanocortin signaling attenuated the metabolic manifestations of cardiac injury-induced cachexia [215]. From the vast amount of research exploring MCR mediation of cachexia it is not always clear whether the manipulations of the melanocortin system, either genetically or pharmacologically, attenuate the metabolic and anorexic effects of cachexia simply by engaging competing orexigenic responses (food appetitive/consummatory behaviors) or by actual direct blockade of signaling responses of the disease state on the melanocortin system. Nonetheless, it is clear from both mouse and rat studies that the melanocortin system holds promise in the potential treatment of disease-induced cachexia.

While various ligands are being developed to target the MC3/4R in the hope of treating obesity, none have so far emerged with FDA approval. It is clear that the overconsumption of calories by obese humans is not driven by energy depletion, but instead likely involves disrupted regulation of hedonic feeding. Principal among the limitations that need to be overcome before melanocortin treatments will be effective in treating obesity is the lack of knowledge about which POMC- and MCR-expressing nuclei are most involved in modulating hedonic feeding. Zhang et al. [54] showed that POMC gene-transfer to the NTS caused mild anorexia, persistent weight loss, improved insulin sensitivity, and increased propensity for voluntary wheel running in the dietary obese rats; similar intervention in the ARC only had minimal physiological and metabolic impacts. The authors speculated that both the ARC and NTS are important brain sites for regulating caloric need (chow consumption), whereas the NTS POMC

neurons may play more of a role in hedonic feeding (i.e., high fat diet consumption) compared to the ARC. The notion of limited involvement of hypothalamic melanocortin signaling in hedonic feeding driving obesity is directly challenged by the report of Garza et al. [93], showing that local knockdown of MC4R by AAV-shRNA interference in rats results in hyperphagia driven increase in body weight gain when rats were maintained on high fat diet (similar effects were not observed when maintained on chow). Unfortunately, less research has been conducted in the mouse on which population(s) of MCR-expressing neurons is involved with hedonic feeding. Part of this limitation comes from the genetic approaches classically used in examining the melanocortin system in energy balance regulation, namely $MC4R^{-/-}$ mice and mice with genetic manipulations within POMC neurons. Both these genetic strategies affect neuronal systems outside of the hypothalamus, in particular the NTS. Future approaches should be taken in both the rat and the mouse to explore the role of MCR signaling in extra-hypothalamic and extra-brainstem structures (e.g., ventral tegmental area, hippocampus, nucleus accumbens) in food intake regulation, especially with regard to hedonic or palatable food intake regulation.

Summary

Without question, our picture of melanocortin regulation in energy balance has advanced considerably over the past two decades. Rodent models have provided evidence of distributed sites of neuronal control as well as the identification of numerous endogenous ligands, receptors, transcription factors, and intracellular signals which illustrate the complexity of the melanocortin pathway as a whole. From this perspective, understanding the etiology and pathogenesis of human diseases characterized by chronic disturbances of food intake and energy expenditure, such as obesity, requires an appreciation of the complexity of the central melanocortin system, and an understanding of how melanocortin effectors function to control energy balance and ultimately, body weight.

References

1. Pi-Sunyer X (2009) The medical risks of obesity. Postgrad Med 121(6):21–33
2. Cattaneo A et al (2010) Overweight and obesity in infants and pre-school children in the European Union: a review of existing data. Obes Rev 11(5):389–398
3. Flegal KM et al (2010) Prevalence and trends in obesity among US adults, 1999–2008. Jama 303(3):235–241
4. Ng SW, et al. (2010) The prevalence and trends of overweight, obesity and nutrition-related non-communicable diseases in the Arabian Gulf States. Obes Rev (in press)
5. Belgardt BF, Bruning JC (2010) CNS leptin and insulin action in the control of energy homeostasis. Ann NY Acad Sci 1212: 97–113
6. Mountjoy KG (2010) Functions for pro-opiomelanocortin-derived peptides in obesity and diabetes. Biochem J 428(3): 305–324
7. Farooqi IS, O'Rahilly S (2005) Monogenic obesity in humans. Annu Rev Med 56:443–458
8. Mountjoy KG (2010) Distribution and function of melanocortin receptors within the brain. Adv Exp Med Biol 681:29–48
9. Bicknell AB (2008) The tissue-specific processing of pro-opiomelanocortin. J Neuroendocrinol 20(6):692–699
10. DeBold CR, Nicholson WE, Orth DN (1988) Immunoreactive proopiomelanocortin (POMC) peptides and POMC-like messenger ribonucleic acid are present in many rat nonpituitary tissues. Endocrinology 122(6):2648–2657
11. Gao Q, Horvath TL (2008) Neuronal control of energy homeostasis. FEBS Lett 582(1):132–141
12. Shizume K (1985) Thirty-five years of progress in the study of MSH. Yale J Biol Med 58(6):561–570
13. Grill HJ (2010) Leptin and the systems neuroscience of meal size control. Front Neuroendocrinol 31(1):61–78
14. Grill HJ (2006) Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. Obesity (Silver Spring) 14 Suppl 5:216S–221S
15. Garfield AS et al (2009) Role of central melanocortin pathways in energy homeostasis. Trends Endocrinol Metab 20(5): 203–215
16. Challis BG et al (2004) Mice lacking pro-opiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3–36). Proc Natl Acad Sci USA 101(13):4695–4700
17. Gropp E et al (2005) Agouti-related peptide-expressing neurons are mandatory for feeding. Nat Neurosci 8(10):1289–1291
18. Graham M et al (1997) Overexpression of Agrt leads to obesity in transgenic mice. Nat Genet 17(3):273–274
19. Luquet S et al (2005) NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science 310(5748):683–685
20. Krude H et al (1998) Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat Genet 19(2):155–157
21. Friedman JM (2009) Leptin at 14 years of age: an ongoing story. Am J Clin Nutr 89(3):973S–979S
22. Zhang Y et al (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372(6505):425–432
23. Aleixandre de Artinano A, Miguel Castro M (2009) Experimental rat models to study the metabolic syndrome. Br J Nutr 102(9):1246–1253
24. Hamann A, Matthaei S (1996) Regulation of energy balance by leptin. Exp Clin Endocrinol Diabetes 104(4):293–300
25. Balthasar N (2006) Genetic dissection of neuronal pathways controlling energy homeostasis. Obesity (Silver Spring) 14 Suppl 5:222S–227S
26. Butler AA (2006) The melanocortin system and energy balance. Peptides 27(2):281–290
27. Chen AS et al (2000) Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat Genet 26(1):97–102
28. Huszar D et al (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88(1):131–141
29. Santini F et al (2009) Melanocortin-4 receptor mutations in obesity. Adv Clin Chem 48:95–109

30. Kaelin CB et al (2008) New ligands for melanocortin receptors. Int J Obes (Lond) 32 Suppl 7:S19–S27
31. Cone RD (2005) Anatomy and regulation of the central melanocortin system. Nat Neurosci 8(5):571–578
32. Pritchard LE, White A (2007) Neuropeptide processing and its impact on melanocortin pathways. Endocrinology 148(9):4201–4207
33. Seidah NG, Chretien M (1992) Proprotein and prohormone convertases of the subtilisin family. Recent developments and future perspectives. Trends Endocrinol Metab 3(4):133–140
34. Seidah NG et al (1991) Cloning and primary sequence of a mouse candidate prohormone convertase PC1 homologous to PC2, Furin, and Kex2: distinct chromosomal localization and messenger RNA distribution in brain and pituitary compared to PC2. Mol Endocrinol 5(1):111–122
35. Pan H et al (2006) The role of prohormone convertase-2 in hypothalamic neuropeptide processing: a quantitative neuropeptidomic study. J Neurochem 98(6):1763–1777
36. Ollmann MM et al (1997) Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science 278(5335):135–138
37. Quillan JM et al (1998) A synthetic human Agouti-related protein-(83–132)-NH2 fragment is a potent inhibitor of melanocortin receptor function. FEBS Lett 428(1–2):59–62
38. Li JY et al (2000) Agouti-related protein-like immunoreactivity: characterization of release from hypothalamic tissue and presence in serum. Endocrinology 141(6):1942–1950
39. Creemers JW et al (2006) Agouti-related protein is posttranslationally cleaved by proprotein convertase 1 to generate agouti-related protein (AGRP)83–132: interaction between AGRP83–132 and melanocortin receptors cannot be influenced by syndecan-3. Endocrinology 147(4):1621–1631
40. Reizes O et al (2001) Transgenic expression of syndecan-1 uncovers a physiological control of feeding behavior by syndecan-3. Cell 106(1):105–116
41. Reizes O et al (2003) Syndecan-3 modulates food intake by interacting with the melanocortin/AgRP pathway. Ann NY Acad Sci 994:66–73
42. Zheng Q et al (2010) Enhanced anorexigenic signaling in lean obesity resistant syndecan-3 null mice. Neuroscience 171(4):1032–1040
43. Takahashi KA, Cone RD (2005) Fasting induces a large, leptin-dependent increase in the intrinsic action potential frequency of orexigenic arcuate nucleus neuropeptide Y/Agouti-related protein neurons. Endocrinology 146(3):1043–1047
44. Palkovits M, Eskay RL (1987) Distribution and possible origin of beta-endorphin and ACTH in discrete brainstem nuclei of rats. Neuropeptides 9(2):123–137
45. Grill HJ, Hayes MR (2009) The nucleus tractus solitarius: a portal for visceral afferent signal processing, energy status assessment and integration of their combined effects on food intake. Int J Obes (Lond) 33 Suppl 1:S11–S15
46. Ritter RC (2004) Gastrointestinal mechanisms of satiation for food. Physiol Behav 81(2):249–273
47. Smith GP (1996) The direct and indirect controls of meal size. Neurosci Biobehav Rev 20(1):41–46
48. Rinaman L et al (1989) Ultrastructural demonstration of a gastric monosynaptic vagal circuit in the nucleus of the solitary tract in rat. J Neurosci 9(6):1985–1996
49. Hayes MR et al (2010) Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. Cell Metab 11(1):77–83
50. Skibicka KP, Grill HJ (2009) Hindbrain leptin stimulation induces anorexia and hyperthermia mediated by hindbrain melanocortin receptors. Endocrinology 150(4):1705–1711
51. Skibicka KP, Grill HJ (2009) Hypothalamic and hindbrain melanocortin receptors contribute to the feeding, thermogenic, and cardiovascular action of melanocortins. Endocrinology 150(12):5351–5361
52. Huo L, Grill HJ, Bjorbaek C (2006) Divergent regulation of proopiomelanocortin neurons by leptin in the nucleus of the solitary tract and in the arcuate hypothalamic nucleus. Diabetes 55(3):567–573
53. Ellacott KL, Halatchev IG, Cone RD (2006) Characterization of leptin-responsive neurons in the caudal brainstem. Endocrinology 147(7):3190–3195
54. Zhang Y et al (2010) Pro-opiomelanocortin gene transfer to the nucleus of the solitary track but not arcuate nucleus ameliorates chronic diet-induced obesity. Neuroscience 169(4):1662–1671
55. Li G et al (2007) Melanocortin activation of nucleus of the solitary tract avoids anorectic tachyphylaxis and induces prolonged weight loss. Am J Physiol Endocrinol Metab 293(1):E252–E258
56. Blevins JE, Baskin DG (2010) Hypothalamic-brainstem circuits controlling eating. Forum Nutr 63:133–140
57. Kaelin CB et al (2004) Transcriptional regulation of agouti-related protein (Agrp) in transgenic mice. Endocrinology 145(12):5798–5806
58. Balthasar N et al (2004) Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. Neuron 42(6):983–991
59. van de Wall E et al (2008) Collective and individual functions of leptin receptor modulated neurons controlling metabolism and ingestion. Endocrinology 149(4):1773–1785
60. Shi H et al (2010) The roles of leptin receptors on POMC neurons in the regulation of sex-specific energy homeostasis. Physiol Behav 100(2):165–172
61. Shi H et al (2008) Sexually different actions of leptin in proopiomelanocortin neurons to regulate glucose homeostasis. Am J Physiol Endocrinol Metab 294(3):E630–E639
62. Huo L et al (2009) Leptin-dependent control of glucose balance and locomotor activity by POMC neurons. Cell Metab 9(6):537–547
63. Hill JW et al (2010) Direct insulin and leptin action on proopiomelanocortin neurons is required for normal glucose homeostasis and fertility. Cell Metab 11(4):286–297
64. Myers MG, Cowley MA, Munzberg H (2008) Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 70:537–556
65. Kievit P et al (2006) Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. Cell Metab 4(2):123–132
66. Banno R et al (2010) PTP1B and SHP2 in POMC neurons reciprocally regulate energy balance in mice. J Clin Invest 120(3):720–734
67. Bates SH et al (2003) STAT3 signalling is required for leptin regulation of energy balance but not reproduction. Nature 421(6925):856–859
68. Bates SH et al (2004) LRb-STAT3 signaling is required for the neuroendocrine regulation of energy expenditure by leptin. Diabetes 53(12):3067–3073
69. Buettner C et al (2006) Critical role of STAT3 in leptin's metabolic actions. Cell Metab 4(1):49–60
70. Xu AW et al (2007) Inactivation of signal transducer and activator of transcription 3 in proopiomelanocortin (Pomc) neurons causes decreased pomc expression, mild obesity, and defects in compensatory refeeding. Endocrinology 148(1):72–80
71. Ernst MB et al (2009) Enhanced Stat3 activation in POMC neurons provokes negative feedback inhibition of leptin and insulin signaling in obesity. J Neurosci 29(37):11582–11593

72. Baskin DG et al (1999) Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. Brain Res 848(1–2):114–123
73. Woods SC et al (1979) Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 282(5738):503–505
74. Havrankova J, Roth J, Brownstein M (1978) Insulin receptors are widely distributed in the central nervous system of the rat. Nature 272(5656):827–829
75. Marks JL et al (1990) Localization of insulin receptor mRNA in rat brain by in situ hybridization. Endocrinology 127(6): 3234–3236
76. Werther GA et al (1987) Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. Endocrinology 121(4):1562–1570
77. Bruning JC et al (2000) Role of brain insulin receptor in control of body weight and reproduction. Science 289(5487):2122–2125
78. Konner AC et al (2007) Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metab 5(6):438–449
79. Lin HV et al (2010) Divergent regulation of energy expenditure and hepatic glucose production by insulin receptor in agouti-related protein and POMC neurons. Diabetes 59(2):337–346
80. Cantley LC (2002) The phosphoinositide 3-kinase pathway. Science 296(5573):1655–1657
81. Hill JW et al (2009) Phosphatidyl inositol 3-kinase signaling in hypothalamic proopiomelanocortin neurons contributes to the regulation of glucose homeostasis. Endocrinology 150(11): 4874–4882
82. Al-Qassab H et al (2009) Dominant role of the p110beta isoform of PI3 K over p110alpha in energy homeostasis regulation by POMC and AgRP neurons. Cell Metab 10(5):343–354
83. Hill JW et al (2008) Acute effects of leptin require PI3 K signaling in hypothalamic proopiomelanocortin neurons in mice. J Clin Invest 118(5):1796–1805
84. Morton GJ et al (2005) Leptin regulates insulin sensitivity via phosphatidylinositol-3-OH kinase signaling in mediobasal hypothalamic neurons. Cell Metab 2(6):411–420
85. Plum L et al (2006) Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet-sensitive obesity. J Clin Invest 116(7):1886–1901
86. Padilla SL, Carmody JS, Zeltser LM (2010) Pomc-expressing progenitors give rise to antagonistic neuronal populations in hypothalamic feeding circuits. Nat Med 16(4):403–405
87. Robbins LS et al (1993) Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell 72(6):827–834
88. Chen W et al (1997) Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides. Cell 91(6): 789–798
89. Chida D et al (2007) Melanocortin 2 receptor is required for adrenal gland development, steroidogenesis, and neonatal gluconeogenesis. Proc Natl Acad Sci USA 104(46):18205–18210
90. Chida D et al (2009) Characterization of mice deficient in melanocortin 2 receptor on a B6/Balbc mix background. Mol Cell Endocrinol 300(1–2):32–36
91. Gantz I et al (1993) Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. J Biol Chem 268(20):15174–15179
92. Balthasar N et al (2005) Divergence of melanocortin pathways in the control of food intake and energy expenditure. Cell 123(3):493–505
93. Garza JC et al (2008) Adeno-associated virus-mediated knockdown of melanocortin-4 receptor in the paraventricular nucleus of the hypothalamus promotes high-fat diet-induced hyperphagia and obesity. J Endocrinol 197(3):471–482
94. Rowland NE et al (2010) Effect of MTII on food intake and brain c-Fos in melanocortin-3, melanocortin-4, and double MC3 and MC4 receptor knockout mice. Peptides 31(12):2314–2317
95. Fan W et al (2007) Thermogenesis activated by central melanocortin signaling is dependent on neurons in the rostral raphe pallidus (rRPa) area. Brain Res 1179:61–69
96. Sutton GM et al (2005) Melanocortinergic modulation of cholecystokinin-induced suppression of feeding through extracellular signal-regulated kinase signaling in rat solitary nucleus. Endocrinology 146(9):3739–3747
97. Williams DL et al (2002) Behavioral processes underlying the intake suppressive effects of melanocortin 3/4 receptor activation in the rat. Psychopharmacology (Berl) 161(1):47–53
98. Skuladottir GV et al (1999) Long term orexigenic effect of a novel melanocortin 4 receptor selective antagonist. Br J Pharmacol 126(1):27–34
99. Vergoni AV et al (1998) Differential influence of a selective melanocortin MC4 receptor antagonist (HS014) on melanocortin-induced behavioral effects in rats. Eur J Pharmacol 362(2–3):95–101
100. Kask A et al (1998) Selective antagonist for the melanocortin 4 receptor (HS014) increases food intake in free-feeding rats. Biochem Biophys Res Commun 245(1):90–93
101. Benoit SC et al (2000) A novel selective melanocortin-4 receptor agonist reduces food intake in rats and mice without producing aversive consequences. J Neurosci 20(9):3442–3448
102. Hansen MJ, Schioth HB, Morris MJ (2005) Feeding responses to a melanocortin agonist and antagonist in obesity induced by a palatable high-fat diet. Brain Res 1039(1–2):137–145
103. Marsh DJ et al (1999) Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. Nat Genet 21(1):119–122
104. Aponte Y, Atasoy D, Sternson SM (2011) AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. Nat Neurosci 14(3):351–355
105. Wu Q, Boyle MP, Palmiter RD (2009) Loss of GABAergic signaling by AgRP neurons to the parabrachial nucleus leads to starvation. Cell 137(7):1225–1234
106. Wu Q, Palmiter RD (2011) *GABA*ergic signaling by AgRP neurons prevents anorexia via a melanocortin-independent mechanism. Eur J Pharmacol (in press)
107. Murphy B et al (2000) Centrally administered MTII affects feeding, drinking, temperature, and activity in the Sprague-Dawley rat. J Appl Physiol 89(1):273–282
108. Fan W et al (1997) Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385(6612):165–168
109. Butler AA et al (2001) Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat Neurosci 4(6):605–611
110. Rossi J et al (2011) Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. Cell Metab 13(2):195–204
111. Cai G et al (2006) A quantitative trait locus on chromosome 18q for physical activity and dietary intake in Hispanic children. Obesity (Silver Spring) 14(9):1596–1604
112. Bray GA, Inoue S, Nishizawa Y (1981) Hypothalamic obesity. The autonomic hypothesis and the lateral hypothalamus. Diabetologia 20 Suppl:366–377
113. Minokoshi Y, Haque MS, Shimazu T (1999) Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes 48(2):287–291
114. Toda C et al (2009) Distinct effects of leptin and a melanocortin receptor agonist injected into medial hypothalamic nuclei on glucose uptake in peripheral tissues. Diabetes 58(12):2757–2765

115. Haque MS et al (1999) Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. Diabetes 48(9):1706–1712
116. Brito MN et al (2007) Differential activation of the sympathetic innervation of adipose tissues by melanocortin receptor stimulation. Endocrinology 148(11):5339–5347
117. Song CK et al (2008) Melanocortin-4 receptor mRNA expressed in sympathetic outflow neurons to brown adipose tissue: neuroanatomical and functional evidence. Am J Physiol Regul Integr Comp Physiol 295(2):R417–R428
118. Vergoni AV, Poggioli R, Bertolini A (1986) Corticotropin inhibits food intake in rats. Neuropeptides 7(2):153–158
119. Lu XY et al (2003) Interaction between alpha-melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. J Neurosci 23(21):7863–7872
120. Xu B et al (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 6(7):736–742
121. Kernie SG, Liebl DJ, Parada LF (2000) BDNF regulates eating behavior and locomotor activity in mice. EMBO J 19(6):1290–1300
122. Ono M et al (1997) Brain-derived neurotrophic factor reduces blood glucose level in obese diabetic mice but not in normal mice. Biochem Biophys Res Commun 238(2):633–637
123. Wang C et al (2010) Brain-derived neurotrophic factor (BDNF) in the hypothalamic ventromedial nucleus increases energy expenditure. Brain Res 1336:66–77
124. Sauer H et al (1993) Brain-derived neurotrophic factor enhances function rather than survival of intrastriatal dopamine cell-rich grafts. Brain Res 626(1–2):37–44
125. Pelleymounter MA, Cullen MJ, Wellman CL (1995) Characteristics of BDNF-induced weight loss. Exp Neurol 131(2):229–238
126. Snider WD (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77(5):627–638
127. Unger TJ et al (2007) Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. J Neurosci 27(52):14265–14274
128. Bariohay B et al (2009) Brain-derived neurotrophic factor/tropomyosin-related kinase receptor type B signaling is a downstream effector of the brainstem melanocortin system in food intake control. Endocrinology 150(6):2646–2653
129. Xu L et al (2010) TrkB agonist antibody dose-dependently raises blood pressure in mice with diet-induced obesity. Am J Hypertens 23(7):732–736
130. Tsao D et al (2008) TrkB agonists ameliorate obesity and associated metabolic conditions in mice. Endocrinology 149(3):1038–1048
131. Bariohay B et al (2005) Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. Endocrinology 146(12):5612–5620
132. Yu Y, Wang Q, Huang XF (2009) Energy-restricted pair-feeding normalizes low levels of brain-derived neurotrophic factor/tyrosine kinase B mRNA expression in the hippocampus, but not ventromedial hypothalamic nucleus, in diet-induced obese mice. Neuroscience 160(2):295–306
133. Kanoski SE et al (2007) The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. Behav Brain Res 182(1):57–66
134. Michaud JL et al (1998) Development of neuroendocrine lineages requires the bHLH-PAS transcription factor SIM1. Genes Dev 12(20):3264–3275
135. Holder JL Jr et al (2004) Sim1 gene dosage modulates the homeostatic feeding response to increased dietary fat in mice. Am J Physiol Endocrinol Metab 287(1):E105–E113
136. Kublaoui BM et al (2006) SIM1 overexpression partially rescues agouti yellow and diet-induced obesity by normalizing food intake. Endocrinology 147(10):4542–4549
137. Lu D et al (1994) Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371(6500):799–802
138. Michaud JL et al (2001) Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus. Hum Mol Genet 10(14):1465–1473
139. Kublaoui BM et al (2006) Sim1 haploinsufficiency impairs melanocortin-mediated anorexia and activation of paraventricular nucleus neurons. Mol Endocrinol 20(10):2483–2492
140. Tolson KP et al (2010) Postnatal Sim1 deficiency causes hyperphagic obesity and reduced Mc4r and oxytocin expression. J Neurosci 30(10):3803–3812
141. Fazzini E et al (1991) Stereotaxic implantation of autologous adrenal medulla into caudate nucleus in four patients with parkinsonism. One-year follow-up. Arch Neurol 48(8):813–820
142. Olson BR et al (1991) Brain oxytocin receptor antagonism blunts the effects of anorexigenic treatments in rats: evidence for central oxytocin inhibition of food intake. Endocrinology 129(2):785–791
143. Kublaoui BM et al (2008) Oxytocin deficiency mediates hyperphagic obesity of Sim1 haploinsufficient mice. Mol Endocrinol 22(7):1723–1734
144. Oh IS et al (2006) Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 443(7112):709–712
145. Garcia-Galiano D et al (2010) Expanding roles of NUCB2/nesfatin-1 in neuroendocrine regulation. J Mol Endocrinol 45(5):281–290
146. Stengel A et al (2009) Central nesfatin-1 reduces dark-phase food intake and gastric emptying in rats: differential role of corticotropin-releasing factor2 receptor. Endocrinology 150(11):4911–4919
147. Shimizu H et al (2009) Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin-independent mechanism. Endocrinology 150(2):662–671
148. Kohno D et al (2008) Nesfatin-1 neurons in paraventricular and supraoptic nuclei of the rat hypothalamus coexpress oxytocin and vasopressin and are activated by refeeding. Endocrinology 149(3):1295–1301
149. Maejima Y et al (2009) Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. Cell Metab 10(5):355–365
150. Yosten GL, Samson WK (2010) The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin receptor antagonist. Am J Physiol Regul Integr Comp Physiol 298(6):R1642–R1647
151. Price CJ, Samson WK, Ferguson AV (2008) Nesfatin-1 inhibits NPY neurons in the arcuate nucleus. Brain Res 1230:99–106
152. Qu D et al (1996) A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380(6571):243–247
153. Rossi M et al (1999) Investigation of the feeding effects of melanin concentrating hormone on food intake–action independent of galanin and the melanocortin receptors. Brain Res 846(2):164–170
154. McBriar MD et al (2006) Discovery of orally efficacious melanin-concentrating hormone receptor-1 antagonists as antiobesity agents. Synthesis, SAR, and biological evaluation of bicyclo[3.1.0]hexyl ureas. J Med Chem 49(7):2294–2310

155. Ludwig DS et al (1998) Melanin-concentrating hormone: a functional melanocortin antagonist in the hypothalamus. Am J Physiol 274(4 Pt 1):E627–E633
156. Kim RY et al (2005) Dynamic regulation of hypothalamic neuropeptide gene expression and food intake by melanocortin analogues and reversal with melanocortin-4 receptor antagonist. Biochem Biophys Res Commun 329(4):1178–1185
157. Shimada M et al (1998) Mice lacking melanin-concentrating hormone are hypophagic and lean. Nature 396(6712):670–674
158. Morton GJ et al (2004) Increased hypothalamic melanin concentrating hormone gene expression during energy restriction involves a melanocortin-independent, estrogen-sensitive mechanism. Peptides 25(4):667–674
159. Hanada R et al (2000) Differential regulation of melanin-concentrating hormone and orexin genes in the agouti-related protein/melanocortin-4 receptor system. Biochem Biophys Res Commun 268(1):88–91
160. Lopez M et al (2007) Orexin expression is regulated by alpha-melanocyte-stimulating hormone. J Neuroendocrinol 19(9): 703–707
161. Hara J et al (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30(2): 345–354
162. Chemelli RM et al (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98(4):437–451
163. Li AJ, Dinh TT, Ritter S (2008) Hyperphagia and obesity produced by arcuate injection of NPY-saporin do not require upregulation of lateral hypothalamic orexigenic peptide genes. Peptides 29(10):1732–1739
164. Zheng H et al (2002) Neurochemical phenotype of hypothalamic neurons showing Fos expression 23 h after intracranial AgRP. Am J Physiol Regul Integr Comp Physiol 282(6):R1773–R1781
165. Blevins JE et al (2009) Forebrain melanocortin signaling enhances the hindbrain satiety response to CCK-8. Am J Physiol Regul Integr Comp Physiol 296(3):R476–R484
166. Fan W et al (2004) Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. Nat Neurosci 7(4):335–336
167. Vaughan CH et al (2006) Effects of oral preload, CCK or bombesin administration on short term food intake of melanocortin 4-receptor knockout (MC4RKO) mice. Peptides 27(12):3226–3233
168. Atalayer D et al (2010) Food demand and meal size in mice with single or combined disruption of melanocortin type 3 and 4 receptors. Am J Physiol Regul Integr Comp Physiol 298(6): R1667–R1674
169. Skibicka KP, Grill HJ (2008) Energetic responses are triggered by caudal brainstem melanocortin receptor stimulation and mediated by local sympathetic effector circuits. Endocrinology 149(7):3605–3616
170. Ruiter M et al (2010) Increased hypothalamic signal transducer and activator of transcription 3 phosphorylation after hindbrain leptin injection. Endocrinology 151(4):1509–1519
171. Voss-Andreae A et al (2007) Role of the central melanocortin circuitry in adaptive thermogenesis of brown adipose tissue. Endocrinology 148(4):1550–1560
172. Williams DL et al (2003) Brainstem melanocortin 3/4 receptor stimulation increases uncoupling protein gene expression in brown fat. Endocrinology 144(11):4692–4697
173. Zheng H et al (2005) Brain stem melanocortinergic modulation of meal size and identification of hypothalamic POMC projections. Am J Physiol Regul Integr Comp Physiol 289(1):R247–R258
174. Benoit SC et al (2000) Comparison of central administration of corticotropin-releasing hormone and urocortin on food intake, conditioned taste aversion, and c-Fos expression. Peptides 21(3):345–351
175. Olson BR et al (1993) c-Fos expression in rat brain and brainstem nuclei in response to treatments that alter food intake and gastric motility. Mol Cell Neurosci 4(1):93–106
176. Blevins JE, Schwartz MW, Baskin DG (2004) Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. Am J Physiol Regul Integr Comp Physiol 287(1):R87–R96
177. Sawchenko PE, Swanson LW (1982) Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. J Comp Neurol 205(3):260–272
178. Zheng H et al (2005) Melanin concentrating hormone innervation of caudal brainstem areas involved in gastrointestinal functions and energy balance. Neuroscience 135(2):611–625
179. Peyron C et al (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 18(23):9996–10015
180. Roselli-Rehfuss L et al (1993) Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. Proc Natl Acad Sci USA 90(19):8856–8860
181. Fong TM et al (1997) ART (protein product of agouti-related transcript) as an antagonist of MC-3 and MC-4 receptors. Biochem Biophys Res Commun 237(3):629–631
182. Marks DL et al (2006) The regulation of food intake by selective stimulation of the type 3 melanocortin receptor (MC3R). Peptides 27(2):259–264
183. Butler AA et al (2000) A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology 141(9):3518–3521
184. Jegou S, Boutelet I, Vaudry H (2000) Melanocortin-3 receptor mRNA expression in pro-opiomelanocortin neurones of the rat arcuate nucleus. J Neuroendocrinol 12(6):501–505
185. Bagnol D et al (1999) Anatomy of an endogenous antagonist: relationship between Agouti-related protein and proopiomelanocortin in brain. J Neurosci 19(18):RC26
186. Cowley MA et al (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411(6836):480–484
187. Sutton GM et al (2010) Central nervous system melanocortin-3 receptors are required for synchronizing metabolism during entrainment to restricted feeding during the light cycle. FASEB J 24(3):862–872
188. Sutton GM et al (2008) The melanocortin-3 receptor is required for entrainment to meal intake. J Neurosci 28(48):12946–12955
189. Begriche K et al (2009) The role of melanocortin neuronal pathways in circadian biology: a new homeostatic output involving melanocortin-3 receptors? Obes Rev 10 Suppl 2:14–24
190. Berthoud HR et al (2006) Brainstem mechanisms integrating gut-derived satiety signals and descending forebrain information in the control of meal size. Physiol Behav 89(4):517–524
191. Peters JH, Simasko SM, Ritter RC (2006) Modulation of vagal afferent excitation and reduction of food intake by leptin and cholecystokinin. Physiol Behav 89(4):477–485
192. Appleyard SM et al (2005) Proopiomelanocortin neurons in nucleus tractus solitarius are activated by visceral afferents: regulation by cholecystokinin and opioids. J Neurosci 25(14):3578–3585
193. Babic T et al (2009) Phenotype of neurons in the nucleus of the solitary tract that express CCK-induced activation of the ERK signaling pathway. Am J Physiol Regul Integr Comp Physiol 296(4):R845–R854

194. Noetzel S et al (2009) CCK-8S activates c-Fos in a dose-dependent manner in nesfatin-1 immunoreactive neurons in the paraventricular nucleus of the hypothalamus and in the nucleus of the solitary tract of the brainstem. Regul Pept 157(1–3):84–91
195. Blevins JE et al (2003) Oxytocin innervation of caudal brainstem nuclei activated by cholecystokinin. Brain Res 993(1–2): 30–41
196. Grill HJ, Smith GP (1988) Cholecystokinin decreases sucrose intake in chronic decerebrate rats. Am J Physiol 254(6 Pt 2):R853–R856
197. Hayes MR, De Jonghe BC, Kanoski SE (2010) Role of the glucagon-like-peptide-1 receptor in the control of energy balance. Physiol Behav 100(5):503–510
198. Seo S et al (2008) Acute effects of glucagon-like peptide-1 on hypothalamic neuropeptide and AMP activated kinase expression in fasted rats. Endocr J 55(5):867–874
199. Huo L et al (2008) Divergent leptin signaling in proglucagon neurons of the nucleus of the solitary tract in mice and rats. Endocrinology 149(2):492–497
200. Levin BE, Dunn-Meynell AA, Routh VH (2002) CNS sensing and regulation of peripheral glucose levels. Int Rev Neurobiol 51:219–258
201. Levin BE et al (2004) Neuronal glucosensing: what do we know after 50 years? Diabetes 53(10):2521–2528
202. Routh VH (2002) Glucose-sensing neurons: are they physiologically relevant? Physiol Behav 76(3):403–413
203. Dunn-Meynell AA et al (2002) Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. Diabetes 51(7):2056–2065
204. Kang L et al (2004) Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. Diabetes 53(3):549–559
205. Parton LE et al (2007) Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. Nature 449(7159):228–232
206. Wang R et al (2004) The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. Diabetes 53(8):1959–1965
207. Mounien L et al (2010) Glut2-dependent glucose-sensing controls thermoregulation by enhancing the leptin sensitivity of NPY and POMC neurons. Faseb J 24(6):1747–1758
208. Skibicka KP, Grill HJ (2009) Hypothalamic and hindbrain nuclei contribute to the anorexic, thermogenic and cardiovascular action of melanocortins. Endocrinology 150:5351–5361
209. Williams DL, Kaplan JM, Grill HJ (2000) The role of the dorsal vagal complex and the vagus nerve in feeding effects of melanocortin-3/4 receptor stimulation. Endocrinology 141(4):1332–1337
210. Williams DL, Grill HJ, Kaplan JM (2004) Food deprivation after treatment blocks the multiple-day hyperphagic response to SHU9119 administration. Brain Res 996(2):180–186
211. Wan S et al (2008) Presynaptic melanocortin-4 receptors on vagal afferent fibers modulate the excitability of rat nucleus tractus solitarius neurons. J Neurosci 28(19):4957–4966
212. Cheung WW et al (2008) Modulation of melanocortin signaling ameliorates uremic cachexia. Kidney Int 74(2):180–186
213. Cheung W et al (2005) Role of leptin and melanocortin signaling in uremia-associated cachexia. J Clin Invest 115(6): 1659–1665
214. Wisse BE, Schwartz MW, Cummings DE (2003) Melanocortin signaling and anorexia in chronic disease states. Ann NY Acad Sci 994:275–281
215. Scarlett JM et al (2010) Genetic and pharmacologic blockade of central melanocortin signaling attenuates cardiac cachexia in rodent models of heart failure. J Endocrinol 206(1):121–130
216. Krasnow SM, Marks DL (2010) Neuropeptides in the pathophysiology and treatment of cachexia. Curr Opin Support Palliat Care 4(4):266–271
217. Laviano A et al (2008) Neural control of the anorexia-cachexia syndrome. Am J Physiol Endocrinol Metab 295(5):E1000–E1008
218. Wisse BE et al (2001) Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. Endocrinology 142(8):3292–3301
219. Choudhury AI et al (2005) The role of insulin receptor substrate 2 in hypothalamic and beta cell function. J Clin Invest 115(4):940–950
220. Mesaros A et al (2008) Activation of Stat3 signaling in AgRP neurons promotes locomotor activity. Cell Metab 7(3):236–248
221. Plum L et al (2009) The obesity susceptibility gene Cpe links FoxO1 signaling in hypothalamic pro-opiomelanocortin neurons with regulation of food intake. Nat Med 15(10):1195–1201
222. Janoschek R et al (2006) gp130 signaling in proopiomelanocortin neurons mediates the acute anorectic response to centrally applied ciliary neurotrophic factor. Proc Natl Acad Sci USA 103(28):10707–10712
223. Belgardt BF et al (2008) PDK1 deficiency in POMC-expressing cells reveals FOXO1-dependent and -independent pathways in control of energy homeostasis and stress response. Cell Metab 7(4):291–301
224. Mori H et al (2009) Critical role for hypothalamic mTOR activity in energy balance. Cell Metab 9(4):362–374
225. Claret M et al (2007) AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J Clin Invest 117(8):2325–2336
226. Ramadori G et al (2010) SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity. Cell Metab 12(1):78–87