Melanocortin neurons: Multiple routes to regulation of metabolism

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Abstract

The burden of disability, premature death, escalating health care costs and lost economic productivity due to obesity and its associated complications including hypertension, stroke, cardiovascular disease and type 2 diabetes is staggering [1,2]. A better understanding of metabolic homeostatic pathways will provide us with insights into the biological mechanisms of obesity and how to fundamentally address this epidemic [3–6]. In mammals, energy balance is maintained via a homeostatic system involving both peripheral and central melanocortin systems; changes in body weight reflect an unbalance of the energetic state [7–9]. Although the primary cause of obesity is unknown, there is significant effort to understand the role of the central melanocortin pathway in the brain as it has been shown that deficiency of proopiomelanocortin (POMC) [10,11] and melanocortin 4 receptors (MC4R) [12–15] in both rodents and humans results in severe hyperphagia and obesity [16–23]. In this review, we will summarize how the central melanocortin pathway helps regulate body mass and adiposity within a ‘healthy’ range through the ‘nutrient...
sensing’ network [24–28]. This article is part of a Special Issue entitled: Melanocortin Receptors - edited by Ya-Xiong Tao.

Keywords
Central melanocortin pathway; Melanocortin 4 receptors; Metabolism; Proopiomelanocortin neurons; Agouti related peptide neurons

1. Introduction

The central melanocortin pathway consists of neurons that release endogenous melanocortin ligands (ACTH, α-, β-, γ-melanocyte-stimulating hormone (MSH)), five receptors (MC1R, MC2R, MC3R, MC4R and MC5R) [29] and the endogenous melanocortin antagonist/inverse agonist agouti and agouti related peptide (AgRP) [30]. The melanocortin ligands (ACTH and α-, β-, γ-MSH) are derived from POMC, which are produced in POMC neurons [31]. In the central nervous system, POMC neurons are located in the arcuate nucleus of the hypothalamus (ARC) and the nucleus of the solitary tract (NTS) of the brain stem, both of which are involved in the regulation of energy balance [32,33]. In response to caloric sufficiency, POMC neurons are activated resulting in decreased food intake, increased energy expenditure and weight loss [34–37]. In contrast, AgRP is the antagonist/inverse agonist for MC3R and MC4R [38]. AgRP is released by AgRP neurons, coexpressed with Neuropeptide Y (NPY) [39,40]. AgRP neurons are stimulated by the orexigenic hormone-ghrelin, but inhibited by anorexigenic hormones such as serotonin or leptin [38,41–46].

ACTH and α-, β-, γ-MSH are the agonists of the central melanocortin system and bind to five different G-protein-coupled melanocortin receptors (MC1R, MC2R, MC3R, MC4R and MC5R) [30]. Of these five identified melanocortin receptors only MC3R and MC4R are expressed in the CNS and are linked to the regulation of energy balance [47]. Neural MC4R has been shown to regulate satiety signals and modulate glucose and lipid metabolism in the periphery [24]. Central administration of MC4R agonists such as α-MSH decreases food intake and increases energy expenditure resulting in weight loss. Central administration of MC4R antagonists such as AgRP have the opposite effect to α-MSH; they increase food intake, decrease energy expenditure, alter metabolism to promote deposition of adipose mass and suppress systemic thermogenesis resulting in weight gain [8,48–50]. MC4R mutations are the most common cause of monogenic obesity in humans [51]. Similar to human mutation, MC4R knockout mice exhibit hyperphagia, hyperglycemia, hyperleptinemia, and hyperinsulinemia [13]. In contrast, the role of MC3R in the regulation of energy homeostasis is more subtle. MC3R knockout mice only slightly increased adiposity and an accelerated diet-induced obesity. Nonetheless, MC3R expression is important for maintenance of glucose rhythms and lipid metabolism [47].

As mentioned previously, AgRP and POMC in the hypothalamic arcuate nucleus are the two upstream neurons in the central melanocortin pathway. These two upstream neurons integrate and distribute the central or peripheral information from hormonal and neural signals including fatty acids (FA), cholecystokinin (CCK), peptide YY (PYY), leptin, insulin, ghrelin, pituitary adenylate cyclase-activating peptide (PACAP), serotonin, GABA
and glutamate (Fig. 1 and Table 1). We now summarize the current evidence of the roles of these signals within the ‘nutrient sensing’ central melanocortin pathway in maintaining body mass and adiposity within a healthy range.

2. Hormonal signals

2.1. Leptin

Leptin is released from peripheral adipose tissue and has an important role on the regulation of energy homeostasis [89,90]. Leptin binds to the leptin receptor (Ob-Rb) in the ARC [91–95] and stimulates the cellular activity of POMC neurons while inhibiting the cellular activity of NPY/AgRP neurons [33]. Leptin or leptin receptor -deficient rodents and humans are obese due to hyperphagia and reduced energy expenditure [91,96]. The administration of leptin into leptin-deficient mice (ob/ob) can totally rescue hyperphagia and limit obesity, while chronic infusions of leptin have been shown to completely deplete visible adipose tissue [97]. Furthermore, ICV administration of leptin into obese mice increases energy expenditure and reduces food intake [52,53]. ICV administration of non-selective MC4R antagonist SHU9119 inhibited the anorexigenic effects of leptin on obese mice [54,55]. Also, ICV administration of leptin could not rescue hyperphagia in obese mice deficient of MC4R (MC4R−/−) [98,99]. Leptin also plays another crucial role in the regulation of glucose homeostasis by decreasing the synthesis/release of AgRP [56,100–102]. Intriguingly, central administration of leptin resulted in decreased glucose production only if the central melanocortin pathway is prevented by SHU9119 [57]. These results suggest that the central melanocortin pathway is the downstream target of leptin in the regulation of body weight, energy balance and glucose homeostasis.

2.2. Insulin

Insulin, a peptide hormone secreted by the pancreatic β-cells, plays a key role in regulating plasma glucose levels in the periphery. The level of blood glucose and the level of adiposity influence insulin secretions in the short and long term, respectively [103,104]. Insulin in the central nervous system is associated with suppression of food intake and body weight gain. Central administration of insulin will bind to insulin receptors (IR) and mimic a state of energy surplus to inhibit food intake and decrease body weight [53,58]. IR are widely expressed in the CNS while the hypothalamus contains the highest expression of IR [105–107]. IR have also been found to be expressed on NPY/AgRP neurons and POMC neurons [108]. Electrophysiological recordings revealed that insulin hyperpolarized NPY/AgRP neurons and depolarized POMC neurons via activation of KATP channels [59,109–112]. Mice lacking IR in the CNS showed mild and sex-specific obesity, hyperleptinemia, and insulin resistance [113]. Deletion of IR alone in AgRP neurons found that insulin action on AgRP neurons was required to suppression of hepatic glucose production [114]. However, deletion of IR alone from POMC neurons failed to influence energy or glucose homeostasis [60,114]. Nonetheless, the melanocortin pathway did have an effect on insulin action, specifically POMC neurons in ARC projected to and acted on distinct MC4R expressing neuronal populations in the intermediolateral nucleus (IML) and dorsal motor nucleus of the vagus (DMV) to decrease insulin secretion or to increase insulin sensitivity separately [115–117]. Additionally, ICV administration of melanocortin agonist (α-MSH) in the third
cerebral ventricle of rats improved insulin sensitivity independent of food intake [61]. Furthermore, it has been shown that MC4R KO mice have impaired insulin tolerance, suggesting the role for MC4R in the regulation of insulin action [62]. One potential mechanism is that the activation of MC4R potentiates insulin-stimulated mTOR signaling via the AMPK pathway [61], inhibits c-Jun N-terminal kinase activity and promotes insulin signaling [118]. These findings suggest the strong interaction between the melanocortin pathway and insulin signaling pathway.

2.3. Fatty acids

Fatty acids (FA) act as a sensor of nutrient availability in the brain to control energy homeostasis [119]. In the hypothalamus, FA detect signals from peripheral tissues to regulate food intake, insulin secretion and hepatic glucose production (HGP) [120,121]. Intracerebroventricular (ICV) infusion of monounsaturated acid oleic acid (OA) or polyunsaturated O-3 docosahexaenoic acid (DHA) into rodents has been shown to increase POMC mRNA expression and decrease NPY mRNA expression, subsequently resulting in release of α-MSH that leads to reduced food intake, body weight and glucose production. The anorexigenic effect of OA is completely abolished if the MC4R antagonist SHU9119 is centrally administered at the same time [63,64,122]. However, a similar result, even after 48 h following centrally administered SHU9119, was not apparent on serum glucose levels – OA continued to have an inhibitory effect on serum glucose production [63,64,122]. Fatty acid synthase (FAS) has been found to co-localize with orexigenic NPY in ARC neurons [123]. The inhibition of FAS C75 activity can significantly decrease food intake and body weight [124,125]. Moreover, this anorexigenic effect is mediated by influencing NPY production in the melanocortin pathway [126]. In conclusion, POMC neurons and AgRP neurons within the hypothalamus indicator the variations in plasma FA levels and their metabolites to regulate energy balance and glucose homoeostasis.

2.4. Cholecystokinin

Cholecystokinin (CCK) is a gut released peptide synthesized by both the gastrointestinal system and the central nervous system [127]. Both peripheral and central administration of CCK lead to the reduction of food intake [65,128,129]. First, following a meal, CCK activates CCKNTS neurons, a subset of nucleus tractus solitaries (NTS) neurons that respond to CCK, to inhibit food intake and reduce meal size [130–135]. Both chemogenetic and optogenetic experiments have shown that this satiating function is mediated by a CCKNTS → PVH pathway. Second, electrophysiological recordings revealed that approximately 23% of PVH MC4R-expressing neurons were excited by CCK-8, indicating that CCK activated the appetite-controlling PVH MC4R-expressing neurons [136]. Third, SHU9119 blocked CCK-induced inhibition of feeding in rats. IP injection of CCK-8 significantly reduced food intake in wild type mice but not in MC4R KO mice [66]. Finally, fourth ventricular administration of MC4R agonist melanotan II (MTII) stimulated phosphorylation of ERK1/2 in NTS while fourth ventricular administration of MC4R antagonist SHU9119 in freely feeding rats restrained the IP injection of CCK-induced phosphorylation of ERK1/2 in the NTS and prevented the reduction of food intake by CCK [137–139]. These results suggest that activation of MC4R is required for CCK-induced suppression of feeding.
2.5. Ghrelin

Ghrelin is an orexigenic peptide and is primarily synthesized in the stomach. Ghrelin binds to growth hormone secretagogue receptor (GHSR) which is mainly located in the medial part of the hypothalamic arcuate nucleus and potently stimulates growth hormone secretion [140–144]. Ghrelin affects energy balance through its involvement in regulation of feeding behavior, glucose and lipid metabolism [67,145–147]. Mice lacking ghrelin receptors are hypophagic and lean when fed a high-fat diet. In addition, these GHSR-null mice exhibit increased locomotor activity and improved glucose homeostasis [148,149]. Ghrelin stimulates food intake and increases fat mass [69,150,151] partly by activating GHSR in the NPY/AgRP neurons within ARC [152,153]. In the ARC, 94% of NPY/AgRP neurons contain GHSR mRNA [154]. Electrophysiological recordings reveal that ghrelin depolarized NPY/AgRP neurons and simultaneously hyperpolarize POMC neurons [155]. Central and peripheral administration of ghrelin increase hypothalamic NPY and AGRP mRNA expression and induce c-fos in NPY/AgRP neurons [69,152]. Therefore, the orexigenic effects of ghrelin are thought to depend on NPY/AgRP neuron release of NPY/AgRP and their subsequent release of GABA inhibiting POMC neurons [70]. However, it is shown that ghrelin cannot stimulate food intake in MC4R null mice, suggesting that the orexigenic effects of ghrelin are partly mediated by the central melanocortin pathway [68]. ICV administration of unacylated ghrelin increases MC4R expression and decreases MC3R expression [156]. In humans, the postprandial suppression of total ghrelin is attenuated in patients with MC4R deficiency compared to lean controls [157], suggesting that the regulation of postprandial ghrelin suppression in humans may involve central melanocortin signaling. These results suggest a critical role of central melanocortin signaling on mediating the orexigenic effects of ghrelin.

2.6. Peptide YY

The peptide YY (PYY) is an anorexigenic gut hormone expressed predominantly in the intestinal L-cells. PYY is implicated in the regulation of energy balance and glucose homeostasis. In response to a meal, PYY is co-secreted with glucagon like peptide 1 [158,159]. PYY knockout mice increases food intake and develop obesity [71,72]. In contrast, PYY overexpressed transgenic mice reduces food intake and are protected against diet-induced obesity [160]. PYY has two main forms, PYY_{1–36} and PYY_{3–36}. PYY_{3–36} represents approximately half of the total postprandial circulating PYY in humans [161]. In humans, peripheral administration of PYY_{3–36} reduces food intake [73,74,162–164]. In diet-induced obese rodent models, peripheral administration of PYY_{3–36} reduce food intake, decreasing body weight and improving insulin sensitivity [165–167]. PYY_{3–36} has a high binding affinity to the NPY receptor Y2 subtype [168,169]. Y2R is shown to be the receptor responsible for mediating the anorectic effect of PYY_{3–36} since PYY_{3–36} is not able to reduce food intake in Y2R-null mice [73]. Peripheral administration of PYY_{3–36} increases POMC mRNA expression and decreases NPY mRNA expression in the ARC [73,75,76] suggesting that the anorexigenic effects of PYY_{3–36} in mice might be mediated by the central melanocortin pathway. Surprisingly, a subsequent study show that MC4R is not essential for the anorexigenic role of PYY_{3–36} [74] since PYY_{3–36} is equally effective in inducing satiety in
wild type and MC4R deficient mice. So, further studies are needed to clarify the interactions between PYY and the melanocortin pathway.

2.7. Pituitary adenylate cyclase-activating peptide

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a peptide originally isolated from the bovine hypothalamus [170]. PAC-AP belongs to the vasoactive intestinal polypeptide/secretin/glucagon family of neuropeptides and is expressed throughout the central nervous system and peripheral tissues including the hypothalamus and pancreas [171]. PACAP and its receptors (PAC1R) abundantly express in the hypothalamus [172,173] and are implicated in the regulation of energy balance in rodents [77,174,175]. Genetic ablation of PACAP in mice is associated with impaired lipid metabolism, carbohydrate intake and brown adipose tissue thermogenesis [78,176,177] while genetic ablation of PAC1R in mice results in impaired insulin response to glucose and reduces glucose tolerance [178]. In addition, ICV administration of PACAP bound to PAC1R increases the expression of POMC mRNA and MC4R mRNA, decreasing food intake and increasing energy expenditure [79]. This effect is attenuated in the genetic ablation of PACAP or PAC1R in mice [174,175]. This effect of PACAP on food consumption is also attenuated by a pretreatment with MC3-R/MC4-R antagonist SHU9119 [79,80]. Thus, these results suggest that PACAP affects energy balance through the melanocortin-dependent pathway.

3. Neural signals

3.1. Serotonin

Serotonin (or 5-hydroxytryptamine, 5-HT) is a multifunctional monoamine neurotransmitter secreted from both peripheral tissues and the brain [179,180]. Serotonin bound to 5-HT2C or 5-HT1B receptors have been shown to inhibit food intake and promote weight loss [181,182]. Pharmacological agents that increase 5-HT activity in the CNS can induce this anorexigenic action of serotonin [183,184]. Global deficiency of 5-HT2C or 5-HT1B receptors in mice resulted in hyperphagia, obesity, impaired glucose homeostasis, and showed attenuated responses to anorexigenic 5-HT drugs [185–188]. Recent studies have shown that the melanocortin pathway is an important downstream mediator of serotonin’s negative action on energy balance. For example, serotonergic terminals made synaptic contacts with arcuate nucleus of the hypothalamus POMC and AgRP neurons [42,189], indicating that the serotonin system was anatomically positioned to influence melanocortin neuron activity [190]. The functional importance of the melanocortin pathway in serotonin’s effects on energy balance has been assessed using pharmacological or genetic inactivation of MC4R. Serotonin or 5-HT2C/5-HT1B receptor agonist mCPP induced MC4R activation by activation of 5-HT2C receptors on POMC neurons and inhibition of 5-HT1B receptors on AgRP neurons [81,82]. Also 5-HT2C receptor-specific agonist D-Fen’s anorectic effects were attenuated in rats and agouti mice pretreated with MC3R/MC4R abbreviation SHU9119 [81,82,190]. Interestingly, mice lacking MC4Rs were not responsive to 5-HT2C receptor agonist-induced hypophagia [188]. Reexpression of MC4Rs only in single-minded homolog 1 neurons in the hypothalamic paraventricular nucleus and in the amygdala was sufficient to restore the hypophagic property of 5-HT2C receptor agonist [188]. These
findings demonstrate that an intact central melanocortin pathway through MC4R is necessary for the anorexigenic action of serotonin [82].

3.2. GABA

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and acts via two different types of membrane GABA receptors: ionotropic (GABA_A) and metabotropic (GABA_B) receptors. Pharmacological studies suggest that both central GABA_A and GABA_B receptor signaling exert prominent influences on feeding and body weight in various brain regions [191–196]. Bilateral lateral hypothalamus injection of GABA_A receptor antagonist picrotoxin acutely evokes feeding while injection of GABA_A receptor agonist muscimol acutely suppresses feeding and decreases body weight [197,198]. In addition, peripheral administration of the GABA_B agonist baclofen significantly reduces food intake and body weight in both diabetic (db/db) and diet-induced obese mice by decreasing NPY expression and increasing POMC expression in ARC [199]. GABA is released by AgRP neurons co-localized with AgRP/NPY–immuno-positive axon terminals which are innervated with local POMC neurons (~70% of POMC neurons expressed GABA_B receptors) in ARC [200–203], suggesting that GABA has a direct inhibitory effect on POMC neurons to rapidly affect the activity of downstream neurons [204]. Additionally, a study of POMC-specific GABA_B receptor-deficient mice shows that GABA_B signaling in POMC neurons protects against obesity and increases insulin sensitivity on the high-fat diet induced mice [205]. Finally, it is shown that GABA released from AgRP neurons bind to GABA_A receptor neurons on the lateral PBN of the hindbrain to regulate appetite and body weight [83,206–208]. Future study using genetic mouse model is necessary to understand the role of GABA on central melanocortin pathway.

3.3. Glutamate

Glutamate is the major excitatory neurotransmitter in the brain [209,210] and plays a role in regulating body weight, food intake and metabolism [84–86,211–213]. The metabotropic glutamate mGluR5 receptor agonist (R,S)-2-chloro-5-hydroxyphenylglycine (CHPG) has been shown to stimulate food intake [214] while the antagonists of glutamatergic NMDA and mGluR5 receptors have been shown to decrease food consumption in a baboon model of binge-eating disorder [215]. Selective disruption of glutamate release in leptin receptor-expressing neurons was found to lead to development of mild obesity due to reduced energy expenditure, suggesting that glutamate release mediates leptin action on energy expenditure [87]. Glutamate increases hypothalamic expression of NPY, POMC and cocaine- and amphetamine-regulated transcript (CART) while reducing AgRP expression [216]. Also, a significant number of vesicular glutamate transporter 2 (VGLuT2)-immunoreactive terminals have been observed on NPY neurons and POMC neurons, suggesting that glutamatergic fibers are located in the ARC [217,218]. These results suggest that glutamate may affect feeding behavior through the melanocortin-dependent pathway. Indeed, selective disruption of glutamate release from paraventricular nucleus (PVH) neurons led to hyperphagia, reduced energy expenditure and rapid development of obesity [88]. Furthermore, it has been shown that conditionally restored MC4R expression only on Sim1 neurons in the background of MC4R-null mice completely reversed the obese phenotype by reversing
hyperphagia. Thus, these results demonstrate that MC4R-expressing glutamatergic neurons in PVH of the hypothalamus are both necessary and sufficient for MC4R control of feeding.

4. Conclusion

Recent advances in the molecular biology and the neuroscience of the melanocortin system using genetic mutations and pharmacological compounds have greatly extended our knowledge of its role in the regulation of energy balance [219,220]. We have provided an overview of the current understanding of the neural systems and the involvement of melanocortins in metabolic homeostasis and the development of obesity. The effect of leptin, CCK, fatty acids, ghrelin and serotonin on energy balance is dependent on melanocortin system. In contrast, the effect of PYY, PACAP and glutamate on energy balance is independent on melanocortin system. We have also raised important questions that will need to be addressed so that we can further understand how the central melanocortin pathway regulates both energy intake and energy expenditure. The development of neuron specific mouse models, CRISPR technologies, optogenetics, chemogenetics, anterograde/retrograde mapping techniques and single cell sequencing has allowed characterization of neuronal or humoral inputs that are important for body weight regulation [221]. Development of primate models will be also necessary to verify the findings from rodents. These future studies will provide important insights to human diseases.

Acknowledgments

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Fig. 1. Participation of the central melanocortin system in metabolic regulation and energy homeostasis.
Table 1

Overview of the effects of administration of neuropeptides on energy balance in control and genetic or pharmacological blockade of MC4-Rs/MC3-Rs.

<table>
<thead>
<tr>
<th>Group of experiments</th>
<th>Body weight</th>
<th>Energy intake</th>
<th>Energy expenditure</th>
<th>HGP</th>
<th>POMC neuron</th>
<th>AgRP neuron</th>
<th>Ref.</th>
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<tr>
<td>Insulin</td>
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<td>[53,58–62]</td>
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↑, increased compared to control group; ↓, decreased compared to control group; =, normal compared to control group. Empty fields, no data available. SHU9119, an MC3-R/MC4-R antagonist.