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Review

Neural melanocortin receptors in obesity and related metabolic disorders ☆

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ABSTRACT

Obesity is a global health issue, as it is associated with increased risk of developing chronic conditions associated with disorders of metabolism such as type 2 diabetes and cardiovascular disease. A better understanding of how excessive fat accumulation develops and causes diseases of the metabolic syndrome is urgently needed. The hypothalamic melanocortin system is an important point of convergence connecting signals of metabolic status with the neural circuitry that governs appetite and the autonomic and neuroendocrine system controling metabolism. This system has a critical role in the defense of body weight and maintenance of homeostasis. Two neural melanocortin receptors, melanocortin 3 and 4 receptors (MC3R and MC4R), play crucial roles in the regulation of energy balance. Mutations in the *MC4R* gene are the most common cause of monogenic obesity in humans, and a large literature indicates a role in regulating both energy intake through the control of satiety and energy expenditure. In contrast, MC3Rs have a more subtle role in energy homeostasis. Results from our lab indicate an important role for MC3Rs in synchronizing rhythms in foraging behavior with caloric cues and maintaining metabolic homeostasis during periods of nutrient scarcity. However, while deletion of the *Mc3r* gene in mice alters nutrient partitioning to favor accumulation of fat mass no obvious role for *MC3R* haploinsufficiency in human obesity has been reported. This article is part of a Special Issue entitled: Modulation of Adipose Tissue in Health and Disease.
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1. Introduction

Obesity is one of the major health issues faced by society in the 21st century [1–3]. Over the last three decades, the number of persons categorized as obese (body mass index of >30 kg/m^2) has risen dramatically in high-income countries and is now also increasing in developing nations. The World Health Organization reported that more people die as a result of conditions resulting from obesity compared to those associated with malnutrition and being underweight. Obesity alters the body's ability to regulate homeostasis and is almost invariably associated with hyperinsulinemia indicating insulin resistance. Obesity is also associated with increased risk of hyperlipidemia and hypertension [4]. Collectively, these conditions are associated with increased prevalence of type 2 diabetes and cardiovascular disease [5]. Strategies for preventing as well as attenuating obesity and its comorbidities are urgently needed.

Simply stated, preventing weight gain requires coordinating calorie intake with energy requirements over time. The homeostatic control of adiposity involves both peripheral and central mechanisms acting in concert [6,7]. For historical reasons, the examination of pathways in the central nervous system initially focused on the hypothalamus. Lesions in the paraventricular (PVN), arcuate (ARC) or ventromedial nuclei of the hypothalamus (VMH) had been observed nearly a century ago to cause hyperphagia and obesity [8,9]. Mouse genetics had the key role in providing leads in the identification of specific neural substrates involved. This review focuses on the central nervous melanocortin system. An abundant literature has developed describing how this system regulates energy balance. First order melanocortin neurons situated primarily in the arcuate nucleus of the hypothalamus (ARC) are regulated by many signals of metabolic status. The transmission of this information about the energy status acts on target neurons expressing two members of the melanocortin receptor family.

This review begins by providing an overview of the melanocortin system and its integration in the neural network involved in the regulation of body weight and metabolic homeostasis. It will then focus on the current understanding of the role of neural melanocortin receptors, the well-known MC4R and the poorly understood MC3R in preventing obesity. Discussions on how the melanocortin system integrates signals from the periphery have been provided in several recent reviews on the regulation of melanocortin neurons that release the endogenous ligands for the melanocortin receptors [10,11].

2. Overview of the melanocortin system

2.1. The melanocortin family: six endogenous ligands and five receptors

The melanocortin receptors are members of the G protein coupled receptor (GPCR) family. Stimulation by melanocortin agonists is associated

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with increased accumulation of cAMP by increased activation of adenylyl cyclase. The five receptors (MC1R to MC5R) were named in order of their cloning [12–24]. The melanocortin receptors have a broad distribution, being expressed throughout the body. MC3R and MC4R are referred as the neural MCRs due to their high expression in the brain, although they also exhibit expression in peripheral tissues [14,25,26].

The melanocortin ligands are produced by proopiomelanocortin (POMC), a preprohormone post-translationally processed by pro-hormone convertases (PC1/PC2) to produce the melanocortins, β-endorphin (β-END), and β- and γ-lipotropin (LPH; Fig. 1). POMC is expressed in the brain, anterior and intermediate lobes of the pituitary, skin and many peripheral organs. The post-translational processing of POMC varies between tissues. Adrenocorticotrophic hormone (ACTH) is produced in the anterior lobe of the pituitary. In the central nervous system, only the melanocyte stimulating hormones (MSH), α-MSH, β-MSH and γ –MSH, are produced with an exception in rodents that lack the N-terminal cleavage site for β-MSH [27].

ACTH, α-MSH, β-MSH, and γ-MSH exhibit agonist activity, albeit with variable degrees of specificity. When agonism is defined as stimulation of cAMP accumulation in cell-based assays, α-, β- and γ-MSH all exhibit agonist activity in cell lines expressing MC1R, MC3R, MC4R and MC5R. However, the MC2R is unique in showing affinity only for ACTH. While γ-MSH exhibits modest selectivity for MC3R, it is still a functional agonist for the other MCRs (Fig. 1) [28,29]. There are three different isoforms of γ-MSH (γ1-, γ2- and γ3-MSH) which may have variable biological activity [30].

The melanocortin receptors are unique when compared to other members of this family in that endogenous antagonists were also identified soon after their cloning. Agouti and Agouti related peptide (AgRP) interact with specific melanocortin receptors to inhibit the activity of MSH. The expression of agouti in mouse is primarily found in the skin, in the cells of the dermal papilla [31] consistent with its role in the regulation of hair-pigment production by melanocyte, but it is also present in testis [32]. The human homolog, agouti signaling protein (ASIP), has been reported to be also expressed in the adipose tissue [33]. AgRP is expressed in the brain, adrenal gland, testis, lung and kidney [34].

Both agouti and AgRP were initially described to function as selective competitive antagonists. Agouti was reported to act as an antagonist for the MC1R and MC4R, while AgRP was reported to function as an antagonist for the MC3R and MC4R [35]. While it is true that agouti and AgRP act as competitive antagonists that prevent binding of MSH, subsequent studies indicated that binding of these peptides to melanocortin receptors results in activation of signaling pathways. Soon after its identification, AgRP was reported to exhibit inverse agonist properties inhibiting the high level of basal activity of MC4Rs [36,37] and MC3Rs [38] in the absence of α-MSH. More recently, it was reported that AgRP could stimulate coupling of MC4R expressed in a hypothalamic neuronal cell line (GT1-7) to the Gi/o subunit [39]. Interestingly, the electrophysiological characterization of the response of neurons to AgRP in the ventromedial hypothalamus has also indicated the involvement of Gi/o-dependent pathways [40]. The relevance of *in vivo* inverse agonism of AgRP is supported by results showing that central administration of AgRP to neuronal specific POMC deficient mice induces a delayed increase in food intake and reduction in oxygen consumption [41]. However, this finding has been questioned with no difference in the obese phenotype of double *Pomc*−/−; *Agrp*−/− mice compared with *Pomc*−/− mice [42,43]. Inverse agonism by agouti at the MC1R has also been suggested to have a functional relevance as it is responsible for the yellow coat color of obese A^y/a mice [44,45].

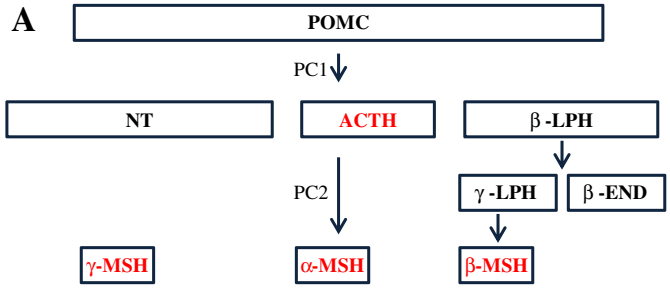
A common feature of several GPCRs is the observation that agonist binding promotes receptor internalization following the recruitment of β-arrestins. The recruitment of β-arrestins and the consecutive internalization of the neural MCRS are observed following binding of either AgRP or α-MSH [46]. Collectively, these findings suggest that MSH and AgRP function as biased agonists and that the regulation of the coupling of MC4R involves both stimulatory or inhibitory G proteins [39,40,47]. It has also been proposed that melanocortin effect on energy expenditure is mediated via Gs, whereas action on food intake involves other signaling [48].

The development of resonance energy transfer techniques has enabled the visualization of GPCR interactions. Much has been learnt on GPCR dimerization/oligomerization, and this is now increasingly accepted as a general phenomenon that results in changes in the biochemical characteristics of GPCRs. Both neural melanocortin receptors have been reported to form heterodimers with other GPCRs [49–53].

2.2. Anatomy of the central melanocortin system

The hypothalamic melanocortin system is a point of intersection in the neurocircuitry connecting appetite and the autonomic and neuroendocrine control of metabolism with signals of metabolic status to defend body weight. Within the brain, two different neuronal populations expressing POMC have been distinguished by their anatomical site. The largest population is located in the hypothalamus, and more specifically in the lateral part of the ARC. This population coexpresses the cocaine amphetamine-related transcript (CART) [54]. A second smaller population is located in the brainstem, in the nucleus of tractus solitarii (NTS). The neuronal population expressing AgRP is restricted to the medial ARC and co-expresses neuropeptide Y (NPY) [55,56]. The pattern of the distribution of AgRP and POMC neuronal projections is similar in the forebrain, however only POMC neurons may send projections to the brainstem [57].

The two distinct neuronal populations described above are considered to have opposite effects on energy homeostasis. POMC neurons via release of MSH inhibit food intake, while AgRP neurons promote food intake. The development of new techniques allowing remote control of neuronal activity either via light-activated ion channels (optogenetic) or via stimulation of "designer" GPCRs (DREADD, Designer Receptors Exclusively Activated by Designer Drug) have helped to clearly establish that acute stimulation of AgRP neurons is sufficient to initiate feeding [58,59]. On the other hand, photic stimulation of channel rhodopsin expressed by POMC neurons decreases food intake [58]. Mice lacking *Pomc* gene products are hyperphagic and obese [60–63]. AgRP over-expressing mice are a mouse model of obesity [35,64]. Unexpectedly, neonatal deletion of AgRP did not produce a lean phenotype [65].



B

Receptor	Ligand affinity at receptor
MC1R	α-MSH =ACTH>β-MSH>γ-MSH
MC2R	ACTH
MC3R	γ-MSH>α-MSH=β-MSH>ACTH
MC4R	α-MSH =β-MSH>ACTH>>γ-MSH
MC5R	α-MSH >ACTH>β-MSH>γ-MSH

Fig. 1. The melanocortin peptides and their receptors. Structure and processing of POMC hormone precursor (A). Affinity of MCRs for the melanocortins (B).

However, a potential long term effect of loss of AgRP since the neonatal period was raised by the reexamination of the *AgRP*−/− mice at an older age that suggested a lean phenotype at 6 month of age [66]. Further studies have shown that postnatal ablation of AgRP neurons has modest to dramatic consequences depending at least partially on the extent of neuronal loss [67–70] that in the most severe cases leads to starvation and death [69]. These results suggest compensatory mechanisms overriding the loss of AgRP in early development. The existence of compensatory mechanisms for loss of AgRP was revived recently by an obese phenotype at 3 months of age in mice where the ablation was performed during the neonatal stage [71].

POMC and AgRP neurons have been described as "first order" neurons in that they are the primary targets of signals communicating energy status, with responses reported for leptin [72–77], insulin [78–81], ghrelin [82–85], serotonin (5-HT) [86–92] and glucose [93,94] (Fig. 2). Among the signals of energy state, leptin has been the most intensively studied. Leptin is a hormone secreted by adipose tissue in proportion to fat mass, and thus reflects the body lipid content [95]. Despite a role in the periphery, it is well-accepted that its primary site of action is the hypothalamus where its full-length receptor, LepRb, is highly expressed in several regions including among the first order neurons [72,96]. Subsets of LepRb-expressing neurons located outside the hypothalamus may also account for leptin action on energy homeostasis [97]. Activation of LepRb on POMC neurons is thought to trigger MSH release while on AgRP neurons it has an inhibitory effect. The population of POMC neurons in ARC is heterogeneous in term of receptor expression, distinct subpopulations of POMC neurons express either LepRb or the insulin receptor, InsR [98,99]; this suggests that signals of energy state (leptin, insulin) are received by different subtypes of POMC neurons.

The release of MSH or AgRP activates second order neurons through melanocortin receptors (Fig. 2). MC3R and MC4R mRNA are localized in different nuclei of the brain [14,26,100–102]. MC4Rs show a broader expression than MC3R being identified in around 100 brain nuclei. Its highest expression is observed in the hypothalamus and the brainstem. MC3Rs have a more restricted pattern of expression, and are expressed in the hypothalamus and limbic structures. The highest levels of expression have been reported in the ARC, VMH, ventral tegmental area (VTA) and the medial habenula (MHb) [14].

Second order neurons expressing MC4Rs include includes oxytocin, thyrotropin-releasing hormone (TRH) and corticotrophin-releasing hormone (CRH) synthesizing neurons in the PVN [102,103]. Preganglionic neurons in the intermediolateral cell column (IML) receiving direct inputs from POMC fibers [54] are another site of MC4R expression. Little is known about the identity of MC3R expressing neurons. Nevertheless, the MC3R is the only melanocortin receptor expressed by POMC and AgRP/NPY neurons in ARC [57,104,105]. MC3R expressed by POMC neurons have been proposed to have an auto-inhibitory role [75,106].

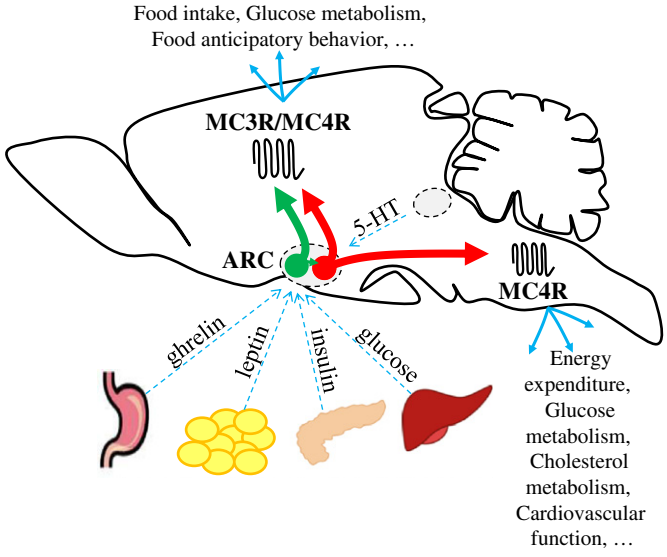


Fig. 2. The hypothalamic melanocortin system. POMC (red) and AgRP (green) neurons in the ARC are referred as "first order" neurons integrating information about the energy state and relaying those information to "second order" neurons expressing MCRs.

3. Genetic evidence: *Mc3r*−/− and *Mc4r*−/− mice studies reveal roles in prevention of obesity

The first study reporting a role for the melanocortin in the regulation of food intake in 1986 suggested that central administration of α-MSH or ACTH1-24 decreased food intake in rat [107]. The cloning of the melanocortin receptors allowed for the development of melanocortin analogs, and many subsequent studies have confirmed and characterized the role of the melanocortin system in the regulation of feeding behaviors and energy balance. Among the melanocortin analogs, the melanocortin agonist analog MTII and agouti mimetic SHU9119 have been the most frequently used. The initial description of their central effect has underscored the tonic inhibitory role of neural melanocortin receptors on feeding [108]. Their affinity for both neural MCRs meant that it was not possible to distinguish between MC3R and MC4R actions. Compounds with selectivity for MC4R, such as HS024 which is a selective MC4R antagonist [109], have been developed. However, the lack of MC3R selective analogs remains to be resolved. Genetic murine models developed for the neural melanocortin receptors *Mc4r* [110] and *Mc3r* [111,112] were a necessary and critical step shedding light on the specific roles of these receptors in energy homeostasis. Results from analyzing the phenotype of these knockouts suggest that both neural MCRs are important for maintaining energy homeostasis. *Mc3r* and *Mc4r* have non redundant roles in the regulation of energy balance as emphasized by the more severe obese phenotype associated with loss of both receptors (double knock-out *Mc3r-Mc4r*) compared to each single knockout mice [112].

3.1. *Mc4r*−/− mice

The connection between the Mc4r and the obese phenotype of A^y/a (yellow agouti) was first hypothesized following the observation that agouti is a functional Mc4r antagonist [113]. Deletion of most of the *Mc4r* coding region in mice was subsequently shown to produce a similar obesity syndrome comprised of maturity-onset weight gain and increased longitudinal growth [110]. *Mc4r*−/− mice also exhibited hyperphagia, hyperinsulinemia, hyperglycemia, and hyperleptinemia relative to wild-type controls, with heterozygous carriers exhibiting an intermediate phenotype [110]. However, the severe diabetes initially observed in *Mc4r*−/− mice on a mixed background was not observed following the back crossing of the null allele onto the C57BL/6J background [114,115], suggesting a significant effect of genetic background. Homozygous carriers of null mutations in the human population while severely obese and hyperinsulinemic also do not exhibit frank type 2 diabetes, indicating that loss of this receptor does not cause diabetes *per se* [116,117].

The *Mc4r*−/− mouse model has proven to be an essential tool in experiments providing information on functional selectivity. A key example was the finding by Marsh and colleagues that *Mc4r*−/− mice do not exhibit reduced food intake following intracerebroventricular injections of MTII, suggesting that MC4R are required for this response [118]. They also found that Mc4r also partially mediates the inhibitory effect of leptin on feeding, as the inhibitory effect of leptin on food intake is attenuated in *Mc4r*−/− mice [118]. The interpretation of these experiments is complicated as obesity in *Mc4r*−/− mice results in severe leptin resistance. However, pair fed or young non obese *Mc4r*−/− mice do exhibit a diminished sensitivity to leptin. On an outbred background, *Mc4r*−/− mice also retain the hypophagic response to leptin treatment and lose weight [119]. Moreover, leptin-deficient mice lacking functional Mc4rs (Lep^{ob}/Lep^{ob};*Mc4r*−/−) also

exhibit diminished sensitivity to leptin treatment compared to Lep^{ob}/Lep^{ob} mice [120]. Leptin thus inhibits food consumption by mechanisms independent of Mc4r. This could involve action of other neurotransmitters released by melanocortin neurons (*e.g.*, GABA), as well as actions on non-melanocortin neurons.

The *Mc4r*−/− mouse model has also been used in experiments that investigate the cause of obesity in this model. While hyperphagia is an obvious mechanism, it is not the only cause. Only one study has reported that hyperphagia, and not hypometabolism, is primarily responsible for the early onset of obesity in *Mc4r*−/− mice [121]. However, pair fed *Mc4r*−/− mice still exhibit increased fat mass [122]. Non pair fed *Mc4r*−/− mice consume less oxygen (suggesting reduced metabolic rate) relative to wild-type controls at 8 weeks of age before their body weight diverge. They also exhibit an increased body weight before the appearance of hyperphagia, suggesting that Mc4r is also involved in the regulation of metabolism [122]. Male *Mc4r*−/− mice exhibit a reduced locomotor activity during the night [122]. Moreover, when expressed as percent of body weight, the resting metabolic rate of *Mc4r*−/− mice is reduced relative to age-matched or weight-matched wild-type mice and is insensitive to MTII administration [123]. The Respiratory Exchange Ratio (RER) or respiratory quotient, which is an indicator of substrate preference to fuel metabolism, is increased in *Mc4r*−/− mice in the fed state suggesting reduced fat oxidation [114,123,124].

MC4Rs are not required for sensing nutritional deficits and the rebound response following a negative energy balance [125]. However, Mc4rs play a role in adaption to changes in diet. Following introduction of more palatable diets with higher fat content, wild-type mice respond by reducing the gram quantity of food consumption in order to maintain an isocaloric intake. In contrast, an exacerbation of weight gain of *Mc4r*−/− mice in response to these diets involves increased food consumption. A concomitant increase in feed efficiency (weight gain per kcal ingested) indicated that other metabolic mechanisms are involved. An increase in locomotor activity observed in wild-type mice at the transition to a diet containing more fat was absent in *Mc4r*−/− mice. A defect in diet-induced thermogenesis and a reduced fatty acid oxidation suggested by a higher RER were also observed. Overall, *Mc4r*−/− mice exhibit an impaired acute homeostatic response in situations where a more palatable diet are presented [125], and also exhibit a positive fat balance in situations where dietary fat intake is increased using defined diets where the ratio of fat and carbohydrate content is altered [114].

The development of new transgenic models based on the cre lox system is helping to resolve the roles of different neuronal populations expressing MC4Rs. The Lowell and Elmquist labs have engineered a "lox stop lox" (LoxTB) Mc4r model, inserting a transcriptional blocker in the 5′ untranslated region of the *Mc4r* gene. Its removal by cre mediated recombination allows restoration of Mc4r expression in discrete cell populations [126]. The phenotype of the homozygous null Lox TB MC4R mouse is identical to that early reported for *Mc4r*−/− mice, and can be rescued by restoration of MC4R during embryogenesis. The use of this model has confirmed that the actions of neural Mc4rs are sufficient for governing energy homeostasis. Using the Sim1 promoter to drive cre expression in PVN and medial amygdala neurons, they observed that MC4Rs expressed in those regions are sufficient to rescue hyperphagia associated with loss of MC4R which accounts for 60% of the obese phenotype [126]. A restoration of the anorectic effect of MTII, but not of its effect to stimulate energy expenditure was also observed in Sim-Cre;LoxTB *Mc4r* mice. This elegant study describes a functional divergence of melanocortin pathways in the control of food intake and energy expenditure (Fig. 3). However, these results need to be interpreted with caution. Post-natal knockdown of *Mc4r* in the adult rat PVN by an AAV-mediated gene silencing results in hyperphagia and excessive weight gain in response to high fat diet but did not alter food intake and body weight on regular chow [127]. MC4Rs outside the PVN in the hypothalamus and in the brainstem have also been implicated in the regulation of appetite [128]. Moreover, MTII administered in the rat PVN stimulates energy expenditure [129]. MC4Rs expressed in other regions may be involved in the regulation of calorie intake; however MC4Rs in the PVN are *sufficient* to only overcome the loss of this receptor in other brain regions with respect to the control of feeding behavior. The Lowell and Elmquist labs subsequently examined the role of MC4Rs expressed by sympathetic preganglionic neurons in the IML, and have suggested that these receptors are required for MC4R actions that result in altered energy expenditure and glucose homeostasis (Fig. 3) [130]. More recently, they have shown that MC4R agonists have opposite effects on the two components of the autonomic nervous system, directly inhibiting the activity of parasympathetic preganglionic neurons in the DMV and exciting the activity of sympathetic preganglionic neurons in the IML (Fig. 3) [131].

3.2. *Mc3r*−/− mice

Mc3r−/− mice exhibit a mild obesity phenotype characterized by increased fat mass, reduced lean mass and reduced body length [111,112]. At six months of age, *Mc3r*−/− mice are hyperleptinemic as well as a hyperinsulinemic but not hyperglycemic [112]. The role of MC3Rs in the regulation of feeding behavior is unclear as emphasized by the discrepancies concerning food intake data from *Mc3r*−/− mice. They were initially described as hypophagic [112] or normophagic on chow diets [111]. More recently, by using an automated system to measure food intake, a slight hyperphagia was observed during the day under high fat diet [124] and hypophagia has been observed during the night under low fat diet [132]. AgRP administered in the third ventricle in *Mc4r*−/− mice still increases food intake, although with less profound effect when compared to wild-type mice [133]. A similar tendency in another study [134] suggests that AgRP could act via Mc3r to regulate feeding behavior. High doses of MTII when given peripherally or centrally in *Mc4r*−/− mice result respectively in reduced and increased food consumption [135]. The partial anorectic response of both *Mc3r*−/− and *Mc4r*−/− mice following central injection of MTII and its abolition in the double knock-out add evidence for non-redundant role of MC3R and MC4R in energy balance [136]. Furthermore, on an outbred background *Mc3r*−/− mice exhibit a delayed anorectic response to leptin [119]. Whereas more studies and the development of specific agonist/antagonist for Mc3r are needed to takes off discrepancies about MC3Rs role in the regulation of food intake *per se*, their involvement in feed efficiency is indicated by their increased fat mass in the absence of hyperphagia.

A recent study provides a potential mechanism, a modest increase in corticosterone levels in the morning during the nadir in the daily rhythms, as a mechanism explaining the obese phenotype [137]. Another possible factor contributing to the obese phenotype of *Mc3r*−/− mice is reduced physical activity which has been observed in their home cage or during the dark phase in cage equipped with wheel [111,112,132,138,139]. Loss of MC3R has also been suggested to reduce "non-resting" energy expenditure, the part of energy expenditure attributable to ambulation and the thermic effect of feeding [138]. However, the role of reduced activity in the obese phenotype is difficult to assess, as a recent study suggested a minor role for home cage activity in energy expenditure in mice not housed at thermoneutrality [140]. Higher RER values have also been reported for *Mc3r*−/− mice indicated altered whole body substrate preference that does not favor fat oxidation [115,124]. Even here, however, discrepancy exists as a more recent study reported a reduced RER [138]. MC3Rs thus appear to have a role in modulating whole body substrate preference; however the contribution of this function to the obese phenotype remains uncertain.

While the phenotype of *Mc3r*−/− mice in the chow fed condition is mild, they are more prone to develop diet-induced obesity as illustrated by their excessive weight gain. This excessive fat accumulation when the dietary fat content is high doesn't result from a defect in diet-induced thermogenesis observed for the *Mc4r*−/− mice, and it

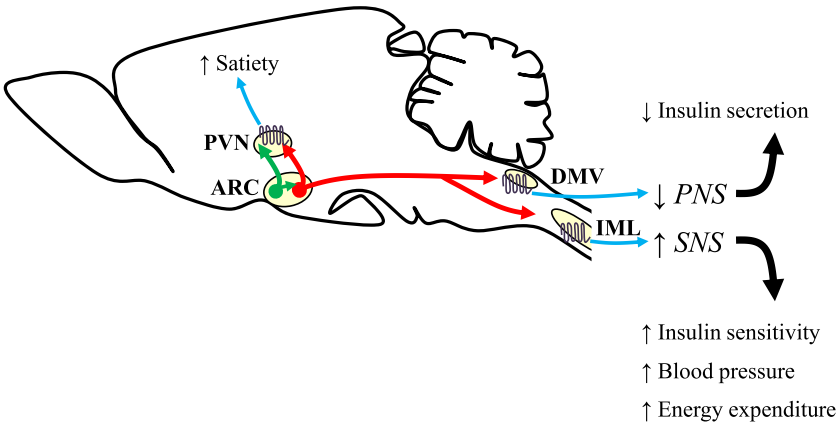


Fig. 3. Divergent pathways in MC4Rs-mediated regulation of energy homeostasis. POMC (red) and AgRP (green) neurons in the ARC send projections to the forebrain, while only POMC neurons may send projections to the brainstem (DMV) and spinal cord (IML). MC4Rs (purple) expressed in the PVN are sufficient to regulate satiety. MC4Rs expressed on preganglionic neurons in the DMV have an inhibitory effect on parasympathetic tone, while in the IML they exert an excitatory effect on the sympathetic outflow.

is possible that altered nutrient partitioning is the primary mechanism [124,125]. Loss of MC3R also impairs metabolic adaptation to fasting [137]. Fasted *Mc3r*−/− mice fail to increase lipolysis and also exhibit reduced activation of the hypothalamo–pituitary–adrenal axis. The increase in orexigenic peptide within the hypothalamus is also attenuated providing an explanation for the decreased food consumption after reintroduction of the food following a fast [137].

Mc3r−/− mice exhibit attenuated adaptation to a restricted feeding protocol. Food restriction entrains circadian rhythms independently of the master clock located in the suprachiasmatic nucleus [141,142]. This phenomenon has been proposed to represent an optimization of feeding behavior, ensuring peak vigilance when nutrients are available. It is thought to involve a food entrainable oscillator that functions independently of the master clock. Restricted feeding or hypocaloric feeding protocols are commonly used to determine the impact of gene mutation or pharmacological treatment on the ability of food cues to entrain circadian rhythms [143]. They consist respectively of restraining food availability to few hours or providing hypocaloric meals at 24 h intervals during the light phase when mice are least active. Mice submitted to these protocols adapt by increasing wakefulness in the hours preceding food presentation and exhibiting food anticipatory activity (FAA), characterized by a rise in activity prior to meal intake. Remarkably, *Mc3r*−/− mice show impaired metabolic and behavioral adaptation to such protocols [132,138,139,144–146]. *Mc3r*−/− mice fail to exhibit increased wakefulness before food presentation, although they will awaken and consume food following its presentation [132]. FAA monitored using running wheels and infrared beam breaks is reduced in *Mc3r*−/− mice relative to controls [132,138,139] (Fig. 4). The same outcome has been observed in absence of light cues when the mice were placed under constant darkness [144]. In contrast, Mc4rs do not seem to be required [132,146].

We recently developed the Lox TB model for MC3R and confirmed that rescuing expression in early embryogenesis recapitulate the behavioral and metabolic phenotype of the *Mc3r*−/− mice. Unexpectedly, restoration using Nestin-Cre of MC3R expression in the central nervous system had only partial consequence on the obese phenotype associated with loss of MC3R. The increase fat mass, reduced lean mass as well as the exacerbation of diet induced obesity were at best partially rescued [138].This result should be interpret with caution, as Nestin-Cre mice exhibit a mild hypopituitarism [147] which could lead to confounding effect. However, if reproduced using other neural-selective Cre transgenics this finding could indicate a previously underestimated role of peripheral MC3Rs in the defense of body weight.

Rescuing MC3Rs in SF1-expressing neurons in VMH also has only a slight effect on the obese phenotype under chow fed conditions, reducing fat mass. Analysis of data from indirect calorimetry suggests an improved whole metabolism substrate preference but no effect on locomotor activity. At the time of the study was started, the VMH was considered as a valid candidate for the localization of the food entrainable oscillator [148]. However, restoring MC3R expression in the VMH failed to rescue the defective adaptation to restricted feeding associated with loss of MC3R. Our unpublished data suggest that others MC3R expressed in the brain may be sufficient as we observed normal FAA in mice expressing MC3R in the nervous system (NesCre LoxTB *Mc3r*). The characterization of neuronal populations expressing MC3R taking part in this complex behavior should thus provides insights into the neural circuitry involved in this behavioral adaptation to restricted feeding.

4. Autonomic and endocrine functions regulated by MCRs in obesity

The autonomic nervous system regulates metabolic homeostasis and cardiovascular function. Melanocortin neurons are involved in

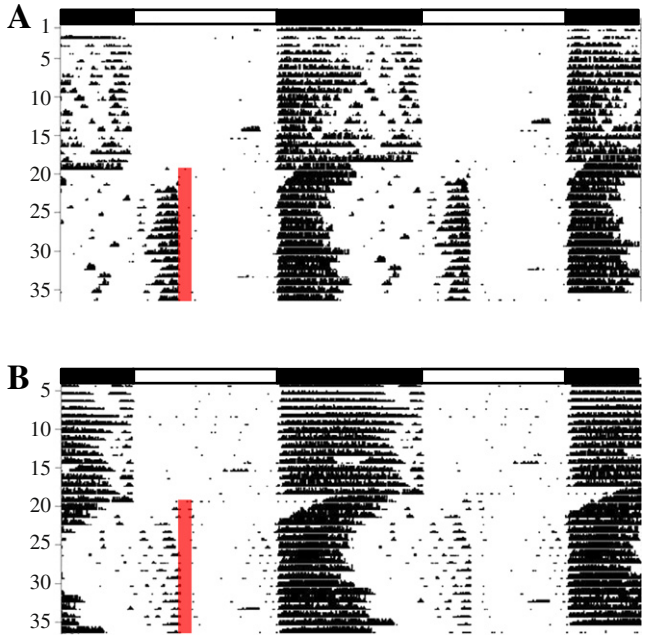


Fig. 4. MC3Rs are required for entrainment to caloric cues. Examples of double-plotted actogram of wheel turns under a 12:12 light-dark cycle in wild-type (A) and loxTB *Mc3r* (B) mice fed *ad libitum* for 17 days and provided once a day with a hypocaloric meal for 19 days. The red line shows the time of food presentation.

modulating autonomic function. Studies using knockout mice suggest that MC4Rs are required for the acute regulation of autonomic function in response to centrally administered melanocortin analogs, with MC3Rs unable to compensate for loss of MC4Rs. MC4Rs are expressed in brain areas known to have autonomic functions including hypothalamic nuclei such as the PVN, the dorsomedial nucleus of the hypothalamus (DMH), and extra-hypothalamic sites [26,101]. In the dorsal motor nucleus of the vagus (DMV) and IML, autonomic preganglionic neurons identified by *Chat* (choline acetyltransferase) mRNA express Mc4r [101]. Viral tracing studies performed by Tim Bartness' laboratory in Siberian hamsters have greatly contributed to the current knowledge about the distribution along the neuroaxis of autonomic neurons expressing MC4R projecting to the interscapular brown adipose tissue (iBAT) [149] and white adipose tissue (WAT) [150]. Expression of MC4R in neurons projecting to the WAT has also been confirmed lately in rat [151]. Additive evidences were provided by pharmacological studies. Administration of MTII dose-dependently increases the sympathetic tone to BAT, as well as renal and lumbar sympathetic nerve activity. SHU9119 when given alone had no effect but is able to block the sympathoexcitation effects of MTII and the leptin induced increase in renal sympathetic nerve activity [152]. Finally, the renal sympathoexcitatory action of MTII, leptin and insulin is blocked in *Mc4r*−/− mice and attenuated in *Mc4r* heterozygote mice [153].

4.1. Adaptive thermogenesis

Brown adipose tissue (BAT) is highly innervated by the sympathetic nervous system and plays a crucial role in adaptive thermogenesis in response to environmental challenges. Melanocortins modulate sympathetic outflow to affect BAT metabolism [152]. Using *Ucp1* mRNA as a readout of sympathoexcitation to BAT, it has been shown that the stimulatory effect of leptin are abrogated in rat when SHU9119 is administered centrally [154] and in *Mc4r*−/− mice [119,122]. Impairment in diet-induced thermogenesis has also been reported in *Mc4r*−/− mice after transition to moderate fat diet [125]. BAT isn't involved as levels of norepinephrine or its turnover in BAT were unaffected in wild-type mice one day after the introduction of the moderate fat diet. Consistently, the increase in oxygen consumption attributable to diet-induced thermogenesis in rats fed a cafeteria diet has been reported not to be determined by increased oxygen consumption by BAT [155,156]. Moreover, the levels of *Ucp1* mRNA in wild-type mice didn't differ between the chow fed conditions and the high-fat diet conditions at thermoneutrality [157]. Howeverthe involvement of BAT in diet-induced thermogenesis is controversial as it has been reported that MC4Rs are required for acute induction of UCP1 in BAT in response to increasing amount of fat in the diet [158]. MTII administered directly in the PVN or in the subzone incerta produces a rise in the iBAT temperature [149,159], whereas HS024, a selective MC4R antagonist, has the opposite effect and inhibits MTII action [159]. AgRP is also potent at regulating iBAT temperature [160] and when administered in the DMH is able to blunt the thermogenic effect of MTII [161].

Beside regulating the activity of the autonomic nervous system, the melanocortins also regulate the hypothalamic-pituitary-thyroid (HPT) axis. Within the hypothalamus, α-MSH and AgRP fibers interact with TRH neurons in PVN [134,162]. MC4Rs are expressed by TRH-synthesizing neurons [102,163], and α-MSH *in vitro* stimulates *Trh* gene expression via activation of MC4R [163] and increases TRH release from hypothalamic explants [164]. *In vivo*, central infusion of α-MSH increases thyrotropin levels [164] and prevents fasting induced suppression of *pro-Trh* gene expression in the PVN [162]. Central infusion of AgRP reproduces the hypothyroid state seen during starvation, but had no effect on circulating T4 levels and *Trh* gene expression in *Mc4r*−/− mice [164,165].

Lately, the importance of Mc4r in the central regulation of thyroid hormone metabolism during fasting has been questioned [166]. By preventing obesity using pair-fed *Mc4r*−/− mice, they found that NPY, but not MC4R, is needed for the fasting induced suppression of the central axis [166]. Furthermore, they highlight a new role for both MC4R and NPY signaling in the regulation of hepatic thyroid hormone metabolism required to suppress TH levels during fasting [166]. At baseline, *Mc4r*−/− mice have normal circulating T4 and TSH levels [165,166] indicating that Mc4r are not required for the basal function of the HPT axis. The first order neurons also contact TRH-synthesizing neurons in human [167]. *MC4R* mutation in human has no impact on free thyroxin levels but a tendency in some patient to have higher thyrotropin levels was observed [116].

4.2. Glucose homeostasis

Insulin resistance resulting in abnormally elevated plasmatic glucose levels is a precursor for type 2 diabetes. Insulin produced by the pancreas acts on glucose homeostasis via two mechanisms, suppressing hepatic glucose production and enhancing glucose disposal. Both mechanisms are modulated by the melanocortin system. Early pharmacological studies using α-MSH and SHU 9119 demonstrated an opposite effect mediated via MC4R on insulin action [168]. Central blockade of MC3R/MC4R increases insulin levels and reduces insulin action in skeletal muscle while increasing glucose utilization in WAT [169]. Interestingly, the results from this study suggested increased insulin signaling in WAT of young "pre-obese" *Mc4r*−/− mice. This outcome suggests that MC4Rs can have markedly different effects on insulin action in peripheral tissues that contribute to altered "nutrient portioning" between tissues.

On a mixed genetic background, chow fed mice lacking MC4R exhibit hyperinsulinemia and hyperglycemia [110,122,126,130,131] while *Mc3r*−/− mice are normoglycemic [111,112]. The insulinemia in *Mc3r*−/− mice is however less robust, since they have been reported to be either hyperinsulinemic [112,138] or normoinsulinemic [111,146] in the fasted and fed states. These data suggest that MC4Rs are the primary modulators of glucose homeostasis in normal conditions. However, a failure of *Mc3r*−/− mice to maintain glucose homeostasis is evident under extreme conditions triggered by increased dietary fat consumption or restricted feeding [90,146]. Fasting hyperinsulinemia is exacerbated by a very high content of fat in the diet in both *Mc3r*−/− and *Mc4r*−/− male mice. *Mc3r*−/− mice have been proposed as a potential model of obesity-induced insulin resistance. Nevertheless, the hyperinsulinemia triggered by high fat diet in *Mc3r*−/− mice is moderate in comparison to *Mc4r*−/− mice, which show the greatest glucose intolerance accompanied by a profound disturbance of insulin receptor signaling [115].

As mentioned above, the severity of the insulin resistant phenotype of pre-obese *Mc4r*−/− mice seems to depend on the strain background [114,170]. Pair feeding of *Mc4r*−/− mice can normalize glucose and insulin levels, suggesting an effect of hyperphagia and subsequently severe obesity on the diabetic phenotype of *Mc4r*−/− mice [122]. Restricted feeding protocol through its association with weight loss improved glucose tolerance in *Mc4r*−/− and A^y/a mice, however *Mc3r*−/− mice become glucose intolerant, hyperglycemic and hyperinsulinemic [146]. This insulin resistance is qualified as mixed since the liver appears to respond normally to elevated insulin. *Mc3r*−/− mice exhibit thus a unique diabetic like phenotype under negative energy balance condition. Treatment of *Mc4r*−/− mice with non-selective melanocortin receptors agonists also improves the hyperinsulinemia suggesting the involvement of other melanocortin receptors in the regulation of insulin sensitivity [171].

LoxTB MCR models have been used to define subsets of neurons expressing MCRs that are involved in glucose homeostasis. The contribution of MC4Rs expressed by pre-autonomic neurons in modulating glucose homeostasis has been suggested by studies using LoxTB *Mc4r* mice. Previously it had been reported that MC4Rs mediate the improvements in glucose homeostasis due to stimulation of 5HT2CR expressed on POMC neurons which are known to project to the IML

[54,90]. Restoring MC4R expression in the parasympathetic nervous system reduces the hyperinsulinemia associated with loss of MC4R, while rescue in the sympathetic nervous system is required to reduce the hyperglycemia and increase glucose disposal [130]. In contrast, deletion of MC4Rs only in cholinergic neurons increases insulin levels suggesting that Mc4r mediated decrease in parasympathetic tone reduce insulin secretion [131].

The central melanocortin system via activation of neural MCRs has also been shown to mediate part of leptin action on glucose homeostasis. In non diabetic rats, upregulation of gluconeogenesis by leptin is dependent on activation of neural MCRs, while leptin mediated downregulation of glycogenolysis involve other pathway [172]. Moreover, leptin antidiabetic actions require a functional melanocortin system. Central blockade of MCRs in streptozotocin induced diabetic rats blocked leptin mediated decrease in blood glucose. However, stimulation of central MCRs is not sufficient to mimic leptin action on glycemia suggesting the interactions of multiple pathways in the regulation of glucose homeostasis via leptin [173].

4.3. Nutrient partitioning and lipid metabolism

Both *Mc3r*−/− and *Mc4r*−/− mice exhibit increased fat mass and adiposity (fat mass as a percent of body weight) compared to wild-type mice. In situation where low fat diets are used, *Mc3r*−/− and *Mc4r*−/− mice have hypertrophied WAT and this is exacerbated under high fat diet [174,175]. Both *Mc3r*−/− and pair-fed *Mc4r*−/− mice show a higher feed efficiency, and in both models a higher RER (at least on the C57BL/6J background) indicates reduced use of fat to fuel metabolism [124]. This nutrient partitioning defect has also been examined during pharmacological studies as central infusion of MTII decreases fat pad weight beyond what can be accounted by a reduction of food intake [176]. Inversely, central blockade of MC3R/MC4R increases fat mass in pair fed rats [169].

Considering the "fatty" phenotype of neural melanocortin null mice, it is not surprising that the melanocortin system may regulate lipid metabolism. Central blockade of MC3R/MC4R promotes lipogenesis and triglycerides storage in WAT, independently of an effect on food intake [169]. In contrast, central stimulation of MC3R/MC4R triggers lipid mobilization in WAT [160,169]. The effects of central MC3R/MC4R on lipid metabolism may be mediated via the sympathetic nervous system, as suggested by the localization of MC4Rs on autonomic neurons projecting to WAT [150,151]. Moreover, blockade or stimulation of central melanocortin receptors respectively decrease or increase the sympathoexcitation to WAT in mice [169], and an increase in norepinephrine turnover in some WAT depots follows central administration of MTII [160]. The absence of an upregulation of triglycerides synthesis in WAT following central MC3R/MC4R blockade in mice lacking all three β-adrenergic receptors has elegantly confirmed the role of the sympathetic nervous system [169].

Central blockade of MC3R/MC4R also alters lipid metabolism in skeletal muscle and liver [169]. The increase in hepatic triglycerides content following intracerebroventricular treatment with SHU9119 is at least partially due to an upregulation of lipogenic enzyme gene expression through the activation of SREBP-1c and PPARγ2 [177], while MTII has the opposite effect in an insulin-independent manner [178]. Hepatic lipid metabolism is also compromised in *Mc4r*−/− mice which exhibit an increased hepatic content in triglycerides [114]. *Mc3r*−/− mice show no difference of fasting liver triglyceride content [174]. Consistent with this last finding, no fatty liver disease was observed in *Mc3r*−/− mice despite an increased adiposity. However, a recent study by our group suggests an altered fatty acid metabolism and a possible fatty liver phenotype in *Mc3r*−/− mice [138].

The melanocortin system may also be involved in regulating cholesterol metabolism through modulation of Mc4r signaling [179]. Elevated levels of cholesterol in the plasma, intestine and liver of *Mc4r*−/− mice have been observed in *Mc4r*−/− mice [180], whereas *Mc3r*−/− mice show normal plasmatic content of cholesterol [179]. Treatment by central infusion of the Mc3r/Mc4r antagonist SHU9119 increase circulating HDL cholesterol, independently of effect on food intake, while MTII infusion in the lateral ventricle produce the opposite effect. Central MC4R blockade affecting plasma cholesterol is vagus nerve dependent, showing that melanocortin action on plasma cholesterol is mediated via the autonomic nervous system [179].

4.4. Cardiovascular functions

Hypertension is one of the metabolic abnormalities associated with obesity and can have major health consequences as this increases the risk to develop cardiovascular diseases. Acute central administration of SHU9119 or MTII has no consequence on heart rate and arterial pressure [152], while several days of MTII infusion results in a rise of arterial pressure [181] prevented by adrenergic blockade [182]. In contrast, chronic blockade of MC3R/MC4R with SHU9119 reduced both arterial pressure and heart rate [181,183] and prevented the hemodynamic responses to leptin [183] through the blockade of MC4R signaling [184]. Rats fed a high fat diet exhibit an increase in arterial pressure and are still sensitive to the cardiovascular action of melanocortin receptors activation [185]. Administration of MTII directly in different brain nuclei expressing MC4R and connected to the sympathetic outflow (PVN, NTS, rostral ventrolateral medulla, parabrachial nucleus, retrochiasmastic area) results in tachycardia [128]. In spite of marked obesity, *Mc4r*−/− mice are not hypertensive and can exhibit lower heart rate [184,186]. Very recently, further evidence for the involvement of the sympathetic nervous system in Mc4r mediated control of blood pressure have been provided; re-expression of MC4R in cholinergic neurons renders the LoxTB *Mc4r* mice hypertensive [131]. *Mc3r*−/− mice are normotensive but can become hypertensive when fed a high sodium diet, due to a failure to respond to the natriuretic actions of γ-MSH [187]. Interestingly, the salt-sensitive hypertension is also accompanied by abnormal glucose metabolism [188]; the underlying mechanism remains unknown but the authors proposed an involvement of the autonomic nervous system [189].

Studies in humans also suggest an important role for human MC4R in blood pressure control [190–192]. Subcutaneous infusion of MC4R agonist in humans leads to an increase in blood pressure and heart rate [190]. MC4R deficiency in human is associated with a reduced prevalence of hypertension [190]. In another study, heart rate and diastolic blood pressure tend also to be lower in humans with MC4R deficiency [191]. Muscle sympathetic nerve activity (MSNA) provides a direct measure of central sympathetic outflow to the vasculature and tends to be lower in MC4R deficient humans. Interestingly, there is an inverse correlation between MSNA with BMI and leptin levels in human *MC4R* mutants [191]. Studies of humans and rodents thus converge in suggesting the requirement of an intact MC4R for obesity-induced hypertension.

5. Human studies: association of *MC4R* mutations with obesity and a lack of a status quo for MC3R

Mutations affecting melanocortin ligands or their processing (POMC, PC1) have been associated with obesity in humans [193], while inversely a polymorphism in the *AgRP* gene has been linked to leanness in humans [194]. The first description of human with POMC deficiency established the role of endogenous ligands of melanocortin receptors in energy balance [195]. Humans lacking POMC gene products exhibit an early-onset obesity, hyperphagia, red hair pigmentation and hypercotisolemia due to the ACTH deficiency. While the altered pigmentation results from the lack of agonism at the MC1R, the ACTH deficiency from the absence of signaling at MC2R, both MC3R and MC4R could contribute to the obese phenotype observed. However, the cumulative human data suggest a preponderant role for human MC4R in the regulation of appetite and body weight. The studies of human mutations have also

contributed to the understanding of the role of the different melanocortin peptides in energy homeostasis. Indeed, mutations resulting in the fusion of β-MSH and β-endorphin reducing the ability to activate the receptor [196] or in the coding sequence of the POMC-derived peptides β-MSH [197,198] impairing the binding of biological activity of the neuropeptides have shed light on a previously underestimated role for β-MSH in regulation of energy homeostasis in humans. A mutation in α-MSH, reducing the binding to MC4R and subsequently affecting its signaling, was present in a lean family member, and was thus insufficient to fully match the criteria to be associated with obesity [197].

A year after the publication describing the *Mc4r*−/− mouse phenotype two frameshift mutations due to either a 4-bp deletion [199] or a 4-bp insertion in the coding sequence of *MC4R* gene [200] that result in a truncated receptor and severe obesity were found in humans. Since those initial observations, over 150 MC4R variants have been reported. *MC4R* mutations are indeed not so rare with a prevalence of up to 5.8% in a cohort of 500 patients with severe childhood obesity [116]. *MC4R* haploinsufficiency is the most common known form of monogenic obesity. Consistently, a recent genome-wide association has shown that common variants near *MC4R* exhibit the strongest association with BMI after variants in *FTO* [201].

MC4R mutations can follow a dominant mode of inheritance of morbid obesity but also recessive pattern of inheritance exists [202]. The phenotype of human with MC4R deficiency is reminiscent of the mouse *Mc4r*−/− phenotype, *i.e.* severe obesity, increased adiposity, hyperphagia, severe hyperinsulinemia, and an increased linear growth [116,202]. Moreover, a gene dosage effect has also been observed in humans since homozygous are more obese than heterozygous *MC4R* mutants [116]. *MC4R* mutations have also been sometimes associated with binge-eating in adults [203] but not always [204]. Hyperphagia seems to be stronger in early childhood with persistent food-seeking behavior as early as 6 months of age [116,202]. Interestingly, enhancement in appetite appears even more pronounced in humans with leptin deficiency [116], as observed in mouse models [125], and consistent with the mediation of the inhibitory effect of leptin on food consumption by other neuropeptides than melanocortins. A *MC4R* polymorphism has also been associated with physical activity [205]. Resting energy expenditure and diet-induced thermogenesis is not affected by *MC4R* mutations [116,203] but the respiratory quotient in patient with MC4R deficiency is higher relative to obese controls, indicating difference in the preference of substrate utilization in humans (reduced fatty acid oxidation).

There are examples of heterozygous carriers of *MC4R* mutations not being obese despite altered receptor function [206]. Indeed, mutations of *MC4R* may not to be 100% effective at causing obesity. Humans with *MC4R* variant retaining residual signaling capacity are less affected than those with a complete loss of function [116]. Moreover, not all the mutations associated with obesity trigger a loss of function, one mutation (D90N) has been suggested to function via a dominant negative effect leading to an impairment of Mc4r signaling due to heterodimerization of mutated and wild-type receptors [49]. Some mutations do not seem to alter the receptor function. The absence of consequence on MC4R signaling can be questioned as in most case the inverse agonism/biased agonism effect of AgRP was not tested. Interestingly, two variants (Val103Ile and Ile125Leu) have instead been linked with a protection against obesity [207,208]. The *in vitro* analysis of the mutated receptors suggests a possible gain of function [209]. Insights about the molecular mechanism of MC4R activation have recently been gained by the association of functional characterization of human mutations with structural modeling [210].

Recent *in vitro* data suggest that aminoglycoside antibiotics can suppress *MC4R* nonsense mutations by allowing the incorporation of a random amino acid at the mutated position [211,212]. However, *in vivo* use of aminoglycoside in a mouse model with a nonsense mutation of *MC4R*, recapitulating the phenotype of the *Mc4r*−/− mice, have failed to show any effect on obesity [212]. Nevertheless, the administration was subcutaneous and aminoglycoside have been shown to have a marginal penetration rate into the brain. Treatment of mice by central administration of aminoglycoside could lead to a different outcome [212].

In contrast to MC4R, the pathogenic role of *MC3R* mutations in human obesity is still unclear. One caveat of MC3R study in human could be the methods used [213]. If we make the parallel with mouse studies, it is tempting to speculate that MC3R might not be clearly associated with obesity as defined by increased body weight but rather by an increase in adiposity which is not correlated with the BMI for the non obese subjects [213]. A link between MC3R and food intake is also unclear in human since both reduction or increase have been reported [214–216]. So far, two mutations of human *MC3R* could be pathogenic, Ile 183 Asn [214,217,218] and Ile 335 Ser [215]. Two common missense variants (Val81Ile and Lys6Thr) initially reported not be associated with obesity but marginally with higher fasting glucose and insulin levels [219] were in later studies [214,216,220]. The analysis of homozygoty for those variants revealed an effect on fat mass and insulin level [214,216,220]. An ethnic effect could also explain part of the divergence as significant association of *MC3R* mutations resulting in a defective receptor with obesity were observed in French children and Italian adult but not in North American adults [221,222].

Targeting neural melanocortin receptors to treat obesity remains a tantalizing prospect [223]. However, a major limitation to targeting this receptor has been the potential side effects associated with the use of such a compound due to the pleiotropic effect of melanocortin ranging from control of blood pressure, allodynia to satiety. Several pharmaceutical companies have developed selective MC4R agonists. To date, these compounds have failed due to modest effects on food intake and weight loss [224] and/or undesired effect on blood pressure [190]. A recent study reported in a diet induced obese non human primate model a transient decrease in food intake accompanied by a weight loss that persists over 8 weeks of treatment with a MC4R agonist, BIM-22493. This compound appears promising since it doesn't affect cardiovascular function [225]. A previous study in mice has characterized the effect of BIM-22493 on energy homeostasis and found that functional MC4R is required for weight loss but not for improved glucose homeostasis [171], suggesting that targeting more MCRs than MC4R might be beneficial to treat obesity and its related disorders.

6. Conclusion and future directions

Over the last two decades, the melanocortin receptors have been cloned and much has been learned on their critical roles in energy homeostasis. Both MC3Rs and MC4Rs are expressed centrally and play a role in energy balance. The fact that *MC4R* mutations have been clearly associated with obesity in human explains at least in part why more studies have focused on MC4R. We know now that actions of MC4Rs on energy homeostasis are broad including control of lipid and glucose metabolism. MC3Rs have a modest impact on energy balance under normal conditions but their contribution to homeostasis is fully expressed under extreme conditions like periods of plenty or scarcity.

The new genetic models, LoxTB MCRs, developed recently offer a unique tool to decipher the role of the different neuronal population regulating energy homeostasis. The use of the LoxTB *Mc4r* model has been useful for distinguishing divergent role of Mc4r expressing neurons in energy balance. MC4Rs expressed in the PVN and amygdala are sufficient to orchestrate food intake while MC4Rs expressed in the autonomic preganglionic neurons affect in an opposite manner sympathetic and parasympathetic tones to regulate energy expenditure, glucose homeostasis and blood pressure. The identity of MC4Rs critical for the central control of lipid metabolism remains to be established. In addition, our studies using the LoxTB *Mc3r* have begun to dissect MC3Rs action on energy homeostasis and circadian rhythmicity. Restoration of MC3R in the VMH improved metabolic homeostasis but was not

sufficient to rescue the defective behavioral response anticipating food intake associated with loss of MC3R. Unpublished data from our lab suggest that other neuronal populations expressing MC3Rs are involved in the expression of food anticipatory rhythms and further exploration will be required to define the populations involved. Surprisingly, neural MC3Rs are not sufficient to rescue the exacerbation of weight gain when the dietary fat intake is high. However, a caveat in drawing this conclusion is that further studies using neural-selective Cre transgenes is required as it is possible that the Nestin-Cre transgene failed to restore normal MC3R expression in a small subset of neurons which are critical for protecting against diet-induced obesity. However, these observations nevertheless point out a functional divergence between MC3Rs in the regulation of energy homeostasis, and underscore an underestimated role for peripheral MC3Rs in the defense of body weight. Future studies will clarify the function of the remaining subset of MCRs expressing cells.

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