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NEW ASPECTS OF MELANOCORTIN SIGNALING: A ROLE FOR PRCP IN ALPHA-MSH DEGRADATION

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Abstract

The role of the central melanocortin system in the regulation of energy metabolism has received much attention during the past decade since gene mutations of key components in melanocortin signaling cause monogenic forms of obesity in animals and humans. In the arcuate nucleus of the hypothalamus the prohormone proopiomelanocortin (POMC) is posttranslationally cleaved to produce α -melanocyte stimulating hormone (α -MSH), a peptide with anorexigenic effects upon activation of the melanocortin receptors (MCRs). α -MSH undergoes extensive post-translational processing and its in vivo activity is short lived due to rapid degradation. The enzymatic process that controls α -MSH inactivation is incompletely understood. Recent evidence suggests that prolyl carboxypeptidase (PRCP) is an enzyme responsible for α -MSH degradation. As for many key melanocortin peptides, gene mutation of PRCP causes a change in the metabolic phenotype of rodents. This review summarizes the current knowledge on the melanocortin system with particular focus on PRCP, a newly discovered component of the melanocortin system.

Keywords

Hypothalamus; arcuate nucleus; POMC processing; alpha-MSH; hormones; fuel; energy metabolism; Melanocortin receptors; signaling pathways; PRCP

Introduction

Obesity has profound consequences dramatically diminishing both the quality and length of life. Compelling evidence has been gathered to suggest that the underlying cause for fat accumulation in peripheral tissues arises, at least in part, from the central nervous system [18, 65, 73, 97, 109, 122, 188, 191, 203].

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The major role played by the central nervous system was further supported by the discovery in 1994 and 1995 of the gene encoding the adipose signal, leptin, [24, 84, 169, 234], and its receptors [137, 208]. Leptin receptors are expressed predominantly in the ventromedial structure of the hypothalamus, the arcuate nucleus where melanocortin neurons are located [136, 168, 187]. The importance of the melanocortin system in the regulation of energy metabolism was revealed when to better understand the role of various melanocortin receptors in the determination of skin color [148, 149, 181], animals in which melanocortin receptor 4 (MC4R) was ablated became morbidly obese [69, 99]. The melanocortin system comprises of neurons expressing POMC and AgRP (the endogenous antagonist) and neurons expressing melanocortin receptors. Today, the melanocortin system is considered a major regulator of energy metabolism since alterations of many of its components have been shown to affect energy balance [39, 65, 73, 97, 98, 122, 187, 189, 191, 220].

1. Role of the hypothalamus in metabolism regulation

The critical role that the arcuate nucleus melanocortin system (the proopiomelanocortin (POMC) cells, the arcuate nucleus agouti-related protein (AgRP)- neuropeptide Y (NPY)-producing cells) [83], plays in mediating leptin's effect on metabolism provided an exceptionally attractive and simple model of the central feeding center. In this model, activation of the POMC neurons by leptin [45, 62] triggers release of α -melanocyte stimulating hormone (α -MSH) from POMC axon terminals, which in turn, activates melanocortin receptors (MCRs) leading to suppressed food intake and increased energy expenditure. Simultaneously, leptin suppresses the activity of arcuate nucleus NPY/AgRP neurons [45, 62], which otherwise, through the release of AgRP, antagonize the effect of α -MSH on MCRs [162]. The NPY/AgRP system not only antagonizes anorexigenic melanocortin cells at their target sites where MCRs are located, but it also very robustly and directly inhibits POMC perikarya [94, 95]. Both NPY as well as the small inhibitory amino acid neurotransmitter, GABA [45], are involved in this inhibition, which occurs through basket-like synaptic innervations of POMC cells by NPY/AgRP terminals [46, 94]. This unidirectional [94] interaction between the NPY/AgRP and POMC perikarya is of critical significance, because it provides a tonic inhibition of the melanocortin cells whenever the NPY/AgRP neurons are active. Because there is no constitutive direct feedback mechanism from the POMC cells to disengage NPY/AgRP neurons, this simple anatomical constellation may provide the easiest explanation why the baseline blueprint of feeding circuits is more likely to promote feeding than satiety. While this bias towards positive energy balance is a necessity from an evolutionary perspective, it is also a likely contributor to the etiology of metabolic disorders, such as obesity.

Despite the important role of the NPY/AgRP neurons in the regulation of energy homeostasis by the melanocortin system, neither NPY nor AgRP single gene knockout mice [68] nor NPY/AgRP double knockout mice [174] exhibited the expected hypophagic phenotype. Because this could be due to compensatory mechanisms during development, two independent studies examined the role of NPY/AgRP and POMC neurons in mediating adult energy regulation [81, 130]. Both groups used the cell-targeted expression of diphtheria toxin receptors that do not exist in mice, making these animals "immune" to diphtheria toxin-induced necrosis. When these mice, which have a normal development, were injected

with two subsequent doses of diphtheria toxin, the targeted hypothalamic neurons rapidly died resulting in hypophagia in NPY neuron-ablated mice [81, 130] and hyperphagia in POMC neuron-ablated animals [81]. In addition, Luquet and collaborators [13]) found that ablation of NPY neurons during the early postnatal period did not result in an overt metabolic phenotype, arguing that functional effects of degenerated neurons in critical developmental periods may be overcome by reorganization of the circuits.

The PVN has emerged as an important nucleus of the hypothalamus for its role in the regulation of energy balance since a subpopulation of its neurons express MC4R, the target of arcuate projections [149]. The restoration of the MC4R gene selectively in the PVN of MC4R knockout mice by viral or genetic manipulation produced mice displaying lower body weight and reduced food intake compared to the MC4R knockout controls [6]. However, the restoration of the receptor was not sufficient to bring energy expenditure to the level of wild type controls. Thus, this suggests that melanocortin regulation of energy expenditure occurs elsewhere in the hypothalamus or through receptors other than MC4R [6]. In addition, MC4R neurons also express Single-minded 1 (SIM1), a transcription factor and one of only six genes implicated in human monogenic obesity [92]. Haploinsufficiency of SIM1 is associated with hyperphagic obesity and increased linear growth in both humans and mice [139]. Additionally, SIM1 heterozygous mice also show hyperphagia and obesity in response to a high-fat diet [93]. Thus, the phenotype of SIM1 haploinsufficiency is similar to that of agouti yellow (Ay), and MC4R knockout mice, both of which are defective in hypothalamic melanocortin signaling.

While the melanocortin system has emerged as key to the hypothalamic control of energy balance, other hypothalamic systems have also been closely tied to this regulatory mechanism. These include the lateral hypothalamic orexigenic neuronal populations producing either melanin concentrating hormone (MCH) [176] or orexin/hypocretin [49, 185], both of which, while in separate cell populations [19], appear to operate by the classic excitatory neurotransmitter, acetylcholine [33]. The lateral hypothalamus (LH) is considered a “feeding center” since lesions of this area induce hypophagia and weight loss [10, 140].

The discovery and functional characterization of two distinct neuronal populations in the LH that express orexigenic peptides, hypocretin/orexin and MCH, has supported the idea that the LH conveys important signals to promote feeding [11]. MCH and orexin/hypocretin expressing neurons are spatially distinct, but both regulate energy balance, among other functions [19, 61, 185, 205]. Leptin inhibits the expression of MCH and orexin/hypocretin in the LH, inhibits the activity of orexin neurons [22, 55, 193, 208, 223], and decreases excitatory inputs onto these neurons [99]. The mechanisms by which leptin regulates LH function are poorly understood. Projections from leptin responsive POMC- and AgRP-expressing neurons onto LH neurons have been identified. In addition, several neuroanatomical studies showed the presence of leptin receptors in LH neurons suggesting that direct leptin action on these neurons may regulate LH functions. Thus, leptin likely regulates LH functions by multiple mechanisms [63, 64].

Another important hypothalamic nucleus involved in the regulation of energy metabolism is the ventromedial nucleus (VMH). Its implication in the regulation of energy balance was

shown when electrolytic lesions to this region in rats resulted in hyperphagia and obesity [17, 18, 89, 90]. Subsequent studies using pharmacological or chemical lesions have demonstrated that the VMH is a critical region that inhibits feeding and increases metabolism [5, 209]. The VMH was subsequently found to be one of the key regions in the hypothalamus where leptin receptors (LRb) are highly expressed [70, 137]. VMH neurons have been shown to be important in mediating leptin's effect to control energy balance [111]. Animals with a selective deletion of the leptin receptor gene in neurons of the VMH expressing steroid factor-1 (SF-1), a transcription factor important for the development of the VMH [48, 166, 192], are obese and hyperphagic [50, 132].

VMH neurons have also been shown to play a role in the regulation of the activity of POMC neurons of the arcuate nucleus of the hypothalamus [201]. Using laser scanning photostimulation (LSPS) in combination with slice electrophysiology, it has been shown that VMH neurons project to POMC neurons and that these projections are mostly excitatory. Furthermore, food deprivation has been shown to decrease these excitatory inputs onto arcuate POMC cells [201].

The activity of the VMH neurons, on the other hand, is also modulated by projections from the arcuate nucleus. However, the projections from the arcuate to the VMH are less dense compared to those to other nuclei [233]. In addition, the VMH contains MC4R, as well as NPY Y1, Y2 and Y5 receptors [14, 85, 112, 125, 128, 219]. Studies have shown that infusions of NPY into the VMH increase feeding, and fasting is associated with elevated levels of NPY in this region [14, 109]. Furthermore, electrophysiological recordings have shown that VMH neuronal responses to α -MSH are decreased in fasted animals and in animals treated with AgRP prior to sacrifice compared to those of ad libitum fed animals [125].

Brain-derived neurotrophic factor (BDNF), that has been shown to affect the metabolic functions regulated by the VMH, is specifically and abundantly expressed in the VMH. Genetic manipulations of BDNF or its TrkB receptor resulted in obesity in both humans and mice [180, 222]. Interestingly, leptin increases BDNF transcripts [155, 156, 212], whereas fasting decreases them selectively in the VMH, suggesting that the BDNF is a regulatory component of leptin signaling. Because BDNF is known to affect synaptic morphology and function [180], synaptic plasticity of the VMH may be a part of the regulatory mechanism of energy regulation. Notably, the anorectic effects of BDNF are not directly mediated by the melanocortin system since BDNF reduces the body weight and food intake of mice that lack the MC4R [222]. This suggests that the VMH may influence POMC activity by other means, such as classic neurotransmitters and/or plasticity.

The redundant interaction of the melanocortin system with other hypothalamic and extrahypothalamic neuronal systems may occur so that alternative signaling modalities can be utilized if a pathway is blocked. Recent developments also suggest that the flexibility of the hypothalamic center of metabolism is not only due to redundant interconnectivity but is also a consequence of its recently uncovered soft wiring [98, 170].

2. Hypothalamic POMC

The importance of the role of POMC in the regulation of energy metabolism has been well documented by several studies in rodents and observations in humans in which POMC gene mutations are associated with an obese phenotype [28, 38, 118]. The POMC gene is expressed in the brain as well as in the periphery such as in the pituitary gland, immune system and skin [231]. Within the brain, POMC is expressed in the hypothalamus and in the nucleus tractus solitarius (NTS) of the brainstem [232]. The POMC gene encodes several peptides that are the products of a complex post-translational process. The bioactive peptides of the POMC gene include adrenocorticotrophin (ACTH), β -endorphin, α -, β - and γ -melanocyte stimulating hormones (MSH). Five melanocortin receptors (MC) have been cloned so far, MC1-5R. While MC1R, MC2R and MC5R have found to be implicated in the pigmentation in the skin, adrenal steroidogenesis and thermoregulation respectively [30, 37, 181], MC3R and MC4R have been shown to play a major role in the regulation of food intake and energy balance [39, 190]. Both MC3R and MC4R knockout mice, for example, are obese. However, while MC4R-deficient mice are hyperphagic [101], MC3R mice are not [21, 29]. The hypothalamic localization of these 2 receptor subtypes is also quite distinctive. Within the hypothalamus, MC3R is expressed in the POMC neurons of the arcuate nucleus, suggesting that MC3R plays an important role in the feedback mechanism of these neurons [45, 106]. Contrary to MC3Rs, MC4Rs are not expressed in the arcuate nucleus but in other areas of the hypothalamus that are important in the regulation of food intake including the PVN, the dorsomedial hypothalamus (DMH) and the LH [106, 127, 149].

The importance of the role of MC4R in the regulation of energy metabolism has been further established when mutations in the MC4R gene in humans were identified and associated with an obese phenotype [214, 230]. In fact, to date, mutations in MC4R are the most common monogenetic form of human obesity described [31].

The projections of the two components of the melanocortin system, POMC and NPY/AgRP neurons, grossly overlap both within and outside of the hypothalamus [19, 105, 138, 217], although they are rarely very long. The common targets of the NPY/AgRP and POMC projections are the hypothalamic parvicellular PVN [44], the lateral hypothalamic and perifornical areas, all of which express melanocortin receptors MC3/4R [149184]. The interplay between AgRP and α -MSH fibers on melanocortin receptor-expressing neurons in the PVN is suggested to be critical for metabolism regulation [44]. The signals that target the PVN can then be distributed in various directions as the PVN projects to brain stem nuclei, the spinal cord, cortex (via various routes), and thalamus, as well as to the portal vessels connecting the hypothalamus to the anterior pituitary.

Besides the PVN, other areas to which melanocortin neurons project include the medial preoptic area (MPA), the periaqueductal gray (PAG), the nucleus of the solitary tract (NTS) and the parabrachial nucleus (PBN) [19].

An important target area of the mediobasal hypothalamic POMC neurons is the preganglionic sympathetic system in the thoracic segment of the spinal cord [61]. These POMC projections are unopposed by NPY/AgRP efferents [61] and are thought to play an

important role in melanocortin regulation of the sympathetic nervous system [61]. Interestingly, POMC neurons that give rise to this spinal cord projection are not homogeneously distributed within the mediobasal hypothalamus [61]. They are mainly located in the retrochiasmatic area of the hypothalamus and in an area lateral from the arcuate nucleus [61]. In fact, when analyzed closely, the appearance of POMC neurons is not homogenous throughout the mediobasal hypothalamus: within the arcuate nucleus, and specifically in its more medial aspects, POMC neurons are smaller compared to the laterally located, larger melanocortin cells. This enlarged soma size is consistent with neurons that have long projections, such as those POMC cells that project to the spinal cord.

Previous arguments have been made that because of the lack of blood brain barrier in the median eminence of the basal hypothalamus, POMC and NPY/AgRP neurons may be direct targets of circulating metabolic hormones [45], such as leptin ghrelin and insulin. However, the assertion that parts of the melanocortin system in close proximity to the median eminence (Fig. 1) would be outside of the blood brain barrier has never been proven. In a very recent study, the relationship between POMC and NPY/AgRP neurons and capillaries of the arcuate nucleus was analyzed [100]. When examined by electron microscopy, there was no example of either of these perikarya being directly associated with any fenestrated vessels or endothelial cells [100].

3. Regulation of POMC in the Hypothalamus

3.1 Leptin

POMC is a regulator of appetite, energy expenditure and glucose metabolism [39]. Thus, its own regulation must be important for the control of energy homeostasis. The fat-derived hormone leptin is a major regulator of POMC. During fasting, low levels of leptin result in a reduction of POMC mRNA. In addition, leptin receptor-deficient rats (*fa/fa*) [117], leptin deficient (*ob/ob*) and leptin receptor (*db/db*) deficient mice [142] have reduced levels of POMC in the hypothalamus compared to their wild type controls, and leptin replacement to *ob/ob* mice induces an increase in POMC mRNA [8, 117, 142, 190].

AGRP is also directly regulated by leptin in the arcuate nucleus [143]. Both POMC and AGRP neurons express leptin receptor [32, 137] and leptin has been shown to directly increase action potentials in POMC neurons while decreasing the frequency of action potentials in AgRP neurons [45]. The signaling pathway activated by the binding of leptin to its receptors has been the object of many studies that have revealed a complex cascade of events (Fig. 1). Leptin binding to its receptors induces an alteration in the conformation of the receptors which enables the activation of Janus kinase/signal transducers and activators of transcription (STAT) (Fig. 1) as well as mitogen-activated protein kinase (MAPK) signal transduction pathways [213]. Stat 3 increases POMC expression by recruiting histone acetylases to the POMC promoter [114]. In addition, leptin increases the firing rates of POMC neurons via the activation of nonspecific cation channels [45] (Fig. 1). Leptin also induces the expression of suppressor of cytokine signaling (SOCS)-3 mRNA which is thought to act as inducible intracellular negative regulators of leptin signal transduction. In agreement with this, transfection data suggest that SOCS-3 is an inhibitor of leptin signaling [62]. Finally, the protein tyrosine phosphatase PTP1B has been shown to be an important

regulator of leptin signaling in POMC neurons since its deletion in this specific neuronal population reduces diet-induced obesity weight gain and adiposity and increases energy expenditure [7]. On the other hand, specific deletion in POMC neurons of SHP2, a non-receptor tyrosine phosphatase, resulted in increased adiposity, weight gain and decreased energy expenditure [7].

In addition to these intracellular effects, leptin has been shown to induce a rearrangement of the synaptic input organization onto the melanocortin neurons [170]. A differential synaptic input organization has indeed been reported in leptin-deficient *ob/ob* mice compared to their wild type controls. Specifically, in *ob/ob* mice, a significantly lower number of synapses onto POMC neurons was reported [170]. Of those synapses, symmetrical putatively inhibitory synapses onto POMC cells were found to dominate over the asymmetrical excitatory synapses. The opposite was observed on the AgRP neurons [170]. Leptin treatment to *ob/ob* mice rapidly reversed the synaptic density to the levels of wild type mice [170]. In agreement with this, different postsynaptic currents onto POMC and AgRP neurons were also found between these two groups. Although synaptic plasticity may not directly trigger alterations in postsynaptic response, and does not alone explain changes in energy states, it suggests that, depending on peripheral metabolic need, the probability of either activation or inhibition of POMC and AgRP may be rapidly modified by synaptic rearrangements. The understanding of the mechanisms underlying the changes in synaptic plasticity in the face of metabolic states could provide important insights into the regulation of feeding, and potentially into the regulation of other complex behavior.

3.2 Insulin

In addition to leptin, other hormones such as insulin, glucocorticoids, estrogen and thyroid hormones are also regulators of POMC neurons [76, 108, 110, 144, 146, 142, 183] (Fig. 1).

Insulin receptors are expressed in POMC neurons and, like leptin, their activation induces the downstream activation of phosphatidylinositol-3 kinase (PI3K; Fig. 1). Indeed, central injections of a specific PI3K inhibitor block the anorexigenic effect of insulin [159]. However, recent data have challenged the notion that acute intracerebroventricularly injected insulin has an anorectic effect in rats [107].

In addition, a recent study mapping insulin and leptin responsive arcuate POMC neurons found that leptin and insulin are largely segregated in distinct subpopulations of the POMC neurons [218].

Insulin receptor (IR) is a membrane-bound tyrosine receptor. In the CNS, when insulin binds its receptor, it induced the autophosphorylation of IR and the recruitment of IRS proteins and subsequent activation of PI3K and MAPK cascades. Concomitantly, PI3K activation also induces the opening of fewer ATP-sensitive potassium channels (K_{ATP}) thus [196] enabling the increased firing of the POMC neurons [167] (Fig. 1).

PI3K activation generates phosphatidylinositol-3,4,5-triphosphate (PIP₃), which binds phosphoinositide-dependent protein kinase 1 (PDK1) that consequently phosphorylates AKT protein kinase. Activated AKT then enters the nucleus to regulate neuropeptide

expression through the phosphorylation and inactivation of the fork-head box-containing transcription factor of the O subfamily type 1 (FoxO1) [113] which is now excluded from the nucleus allowing POMC transcription (Figure 1) [74, 114, 226]. In support of these findings, mice with a deletion of FoxO1 specifically in POMC neurons have a leaner phenotype due to a significant decrease in food intake with unchanged energy expenditure and locomotor activity [171]. However, no effect on POMC mRNA levels was observed in these mice. On the other hand, FoxO1 inactivation in POMC neurons was found to act on POMC processing by affecting carboxypeptidase E (*Cpe*) mRNA and activity levels [171].

The central effects of insulin also include the regulation of glucose metabolism in the periphery [20]. By inactivating insulin receptors in neurons and glial cells, it has been found that mice are prone to diet-induced obesity, have mild insulin resistance and impaired hepatic glucose production [20]. Central injection of insulin reduces glucose production in the liver, whereas, blocking insulin signaling with an inhibitor of central K_{ATP} channels, diminishes the ability of insulin to suppress peripheral glucose output [160]. This regulation may involve electric transmission via the autonomic nervous system, since transection of the vagal nerve abolishes this effect [172]. Conditional ablation of insulin receptors in either NPY/AgRP or POMC neurons has shown that only AgRP neurons may be important regulators of hepatic glucose production [116]. In addition, targeted knock-ins of insulin receptors in either POMC or AgRP neurons in mice in which insulin receptors are significantly reduced [163] in liver, pancreatic beta cells and several brain areas including the arcuate nucleus (L1 mice) showed that while AgRP regulates hepatic glucose production, POMC neurons regulate energy expenditure and locomotor activity [126]. However, very recent data have shown that concomitant insulin and leptin action in POMC neurons is required to maintain glucose homeostasis and reproductive function [91]. Indeed, by knocking down both leptin and insulin receptors in POMC neurons (Pomc-Cre, *Lepr*(flox/flox) *IR*(flox/flox) mice), mice display systemic insulin resistance, which is distinct from the single deletion of either receptor. In addition, Pomc-Cre, *Lepr*(flox/flox) *IR*(flox/flox) female mice display elevated serum testosterone levels and ovarian abnormalities, resulting in reduced fertility [91]. Finally, recent evidence suggests that central insulin action also regulates fat metabolism [115]. However, the site of action within the brain is still unclear.

3.3 Estrogen

The known effect of estrogen on energy metabolism occurs by regulation of the melanocortin neurons [76–78]. Like leptin, estrogen has been shown to trigger robust synaptic input rearrangement on the POMC neurons of the arcuate nucleus [76] which leads to a corresponding change in the electric properties of these cells. Thus, estrogen regulation of energy homeostasis, like leptin, involves synaptic rearrangement onto the POMC neurons to induce an anti-obese neuronal response. Since both leptin and estrogen are also important regulators of reproduction, a similar mechanism underlying hypothalamic control of fertility may be hypothesized. Whether this involves a direct interaction between the two signals remains to be proved. However, this idea is supported by several data. For example, in females, chronic estrogen withdrawal, as in ovariectomy in rodents and postmenopause in humans, causes leptin resistance, while estrogen replacement prevents this phenotype [2].

Furthermore, estrogen increases leptin-induced STAT3 phosphorylation in the arcuate nucleus [for review see 77], indicating that estrogen may act on a similar target to that of leptin (Fig. 1). Interestingly, estrogen's effect on energy homeostasis and synaptogenesis is independent of leptin and leptin receptors [59, 76, 80]. Thus, this suggests that interaction between estrogen and leptin signaling must bypass leptin receptors and directly target the downstream components.

STAT3 has been reported to be a target of estrogen signaling in cells [13, 194]. Distinct activation mechanisms by which estrogen activates STAT3 were observed. For example, STAT3 is phosphorylated and activated in response to estrogen in cells involving multiple intracellular signal pathways that include MAPK, Src-kinase and PI3K [13, 25] and/or direct physical association between estrogen receptors and STAT3 [34].

Peripheral administration of estrogen induced tyrosine phosphorylation of STAT3 in the hypothalamus in less than 30 minutes. This effect of estrogen on STAT3 tyrosine phosphorylation is JAK independent, since it is effective in leptin receptor knockout mice [76]. Finally, in neural STAT3 knockout (STAT3N^{-/-}) mice, which exhibit an obese and infertile phenotype, highly resembling leptin deficiency [75], estrogen exhibited no effect on reducing body weight. These observations strongly suggest that STAT3 is downstream of estrogen signaling in neurons and is required for the anti-obesity effect of estrogen to occur.

The cross-talk between estrogen and STAT3 takes place, at least in part, through classic estrogen receptors (ERs), the ligand-dependent nuclear transcription factors. A potential role for the recently uncovered membrane estrogen receptors [175] in this matter may not be ruled out; however, it requires more study. Estrogen receptor- α (ER α) has been suggested to be the main estrogen receptor that mediates E2's anti-obesity effect in both sexes [78, 182]. ER α is the dominant estrogen receptor in the arcuate nucleus of the hypothalamus where POMC neurons are located while levels of ER β are negligible [165]. When ER α is knocked out, it causes obesity and infertility in both males and females [87, 88]. While ER α -null animals develop obesity, ER β -null mice are lean. In addition, levels of circulating E2 in obese ER α -null animals are 10 times higher than those in their wild-type littermates [43], revealing estrogen resistance in these mice, and additional administration of E2 in these mice does not reduce either their food intake or their body weight [78]. Finally, estrogen administration to ER α -null mutants does not alter estrogen-induced synaptic plasticity onto the POMC perikarya [76]. Thus, it is reasonable to suggest that estradiol's effect on feeding, metabolism, synaptic plasticity and fertility is mediated by the α -type estrogen receptor and potentially involves STAT3.

3.4 Thyroid Hormones

Thyroid hormone regulation of melanocortin neurons involves the activity of the thyroid hormone activating enzyme, 5' deiodinase type II (DII) in glial cells of the hypothalamus. A differential expression of DII during fasting seems to contribute to the activation of NPY/AgRP and inhibition of α -MSH neurons. During negative energy balance such as fasting, DII mRNA and activity levels are elevated in the arcuate nucleus-median eminence region [51] and this elevation in DII production and activity seems to be regulated by the inverse shift in circulating levels of leptin and corticosterone [41]. Thus, the local hyperthyroidism

[41, 42] caused by DII activation could then affect and alter neuropeptide expression in leptin-responsive arcuate neurons.

The signaling modality by which T3 affects arcuate neurons seems to involve a thyroid hormone regulated mitochondrial protein, uncoupling protein 2 (UCP2; Fig. 1) [42, 121]. UCP2, a protein belonging to a family of mitochondrial anion carrier, is located in the inner membrane of the mitochondria and its primary function is thought to leak hydrogen protons from the intermembrane space to the matrix of the mitochondria [16, 72]. Through this process, UCP2 may affect ATP and superoxide production, and decrease the entry of calcium to the mitochondrial matrix [157].

In the brain, UCP2 is expressed predominantly in neuronal populations of brain stem and hypothalamus involved in the central regulation of autonomic, endocrine, and metabolic processes in both rodents and primates. [53, 96, 179].

The involvement of UCP2 in central metabolic pathways is supported by the finding of an abundant expression of UCP2 in NPY/AgRP [42, 96] and POMC neurons [167]. Thus, thyroid hormone regulation of melanocortin neurons seems to act via a mitochondrial mechanism that by affecting ATP levels and free radical formation will then increase the activity of NPY/AgRP neurons and decrease the activity of POMC cells [3, 54].

3.5 Glucocorticoids

Glucocorticoids play a key function in metabolism regulation by regulating hypothalamic POMC. Many of the genetic models of obesity are characterized by elevated corticosterone levels. In addition, corticosterone withdrawal such as after adrenalectomy (ADX) reduces food intake, fat stores and body weight gain [for review see 47]. It has been shown that ADX induced an increase of POMC mRNA and Fos expression in α -MSH-immunoreactive neurons in rats [183]. In addition, ADX is able to reverse the obese phenotype and restore hypothalamic melanocortin tone in leptin-deficient *ob/ob* mice [133]. ADX has been shown to alter the sensitivity of the central melanocortin system to the effects of AgRP, the melanocortin antagonist [57]. This effect is restored by corticosterone replacement [57]. Glucocorticoids could also have a direct effect on POMC neurons since glucocorticoid receptors are expressed in these neurons and glucocorticoid response elements (GRE) have been identified in the POMC promoter [36, 58]. Recent data [82] suggest that the known orexigenic action of corticosteroids is mediated, at least in part, by synaptic changes and altered excitability of the melanocortin system.

3.6 Circulating nutrients

Nutritional signals have been known to regulate energy balance by hypothalamic neurons sensitive to the metabolic status [145]. Circulating nutrients such as glucose, amino acids and fatty acids, affect POMC neurons. Several metabolic fuel sensors have been shown to be activated in POMC neurons according to the metabolic state of the organism. A considerable amount of work has been devoted to decipher the mechanism by which “nutrient sensing” occurs in the regulation of whole body energy metabolism. A large amount of data has accumulated to suggest that specific mechanisms exist for the “sensing” of the levels of

various circulating nutrients, such as carbohydrates (glucose), fatty- and amino acids, by the brain. These sensors include mammalian target of rapamycin (mTOR) kinase, Sirtuin1 (Sirt 1), AMP-activated protein kinase (AMPK) and glucokinase. In addition to PI3K cascade regulation, FoxO1 has been recently shown to be regulated by a NAD⁺-dependent deacetylase, Sirt1 [23] (Fig. 1). Sirt1 is expressed in POMC neurons and its activity levels are increased by fasting [177]. Cakir and collaborators recently found that the fasting-induced decrease of FoxO1 acetylation and POMC expression are Sirt1-dependent [23]. Furthermore, they also showed that Sirt1 regulates S6 Kinase signaling which has been previously shown to be under the control of mTOR kinase activity [86, 220, 221]. mTOR kinase activity, which is inversely altered by changes in the AMP/ATP ratio, leads to the phosphorylation of its downstream targets including S6K1, S6 ribosomal protein (S6), and eukaryotic initiation factor 4E-binding protein (4E-BP1) [86, 220, 221]. The chronic activation of mTOR specifically in POMC neurons has been found to induce a defect in the development of POMC projections in a cell-autonomous manner as well as an increase in the size of the soma [146]. As mTOR signaling is critical for protein synthesis in general, it is reasonable to assume that alteration in mTOR activity will affect many neuronal parameters such as membrane potential and synaptic events. Although Sirt1 and mTOR seem to regulate POMC neurons, it remains to be established whether they act independently or whether one is upstream to the other. Furthermore, recent data have reported that in peripheral tissues such as muscle, Sirt 1 is regulated by AMPK [26] the activity of which changes inversely to that of mTOR and according to changes in the AMP/ATP ratio. As for Sirt 1, fasting induces an increase of AMPK by increasing its phosphorylation on Thr172 within its catalytic α subunit. The activation of AMPK in the hypothalamus has been shown to be involved in the fatty acid metabolic pathway [120]. Another sensing mechanism specific for glucose that was demonstrated first in pancreatic beta cells [211] and then in hypothalamic neurons [60, 229] is mediated by glucokinase. However, the mechanism by which glucokinase regulates glucose-sensing appears to be independent of ATP as shown by several studies [71, 228].

Any of the aforementioned mechanisms, in turn, must trigger an orchestrated and predictable alteration of the activity of the POMC neuronal circuits that alter behavior as well as autonomic and endocrine functions so that adaption to the changing metabolic milieu can occur. Thus, when one considers fuel sensing, it is from the perspective of whole body responses to circulating carbohydrates, fatty acids or amino acids. The idea that there is a “sensor” of these fuels on neurons of the hypothalamus independent of fuel metabolism has never been proven to exist. In contrast, the utilization of specific fuels by hypothalamic neurons in support of the whole body metabolism of that class of nutrient as a way of “fuel sensing” is more in line with available data. For example, glucose is considered the main driver of POMC neuronal firing [154, 157, 158]. POMC neurons increase their firing when glucose levels rise and this, in turn, affects hepatic glucose homeostasis and insulin action [99, 154, 157, 158]. This is entirely consistent with the putative role of POMC cells as satiety signaling neurons, since at satiety, circulating glucose levels are elevated and POMC neurons exhibit increased firing [159]. In turn, the increase in glucose oxidation induces a rise of free radicals which seems to play an important role as a driving force of POMC neuronal firing [3]. Conversely, during negative energy balance when glucose levels are

lower and circulating fatty acids increase, POMC neurons are silent while NPY/AgRP neurons show increased firing [3]. The intracellular mechanism of fatty acid oxidation occurs without an elevation of ROS levels [3].

4. POMC processing in the hypothalamus

The POMC-derived neuropeptide, α -MSH, is generated by extensive posttranslational processes that involve several enzymes such as the prohormone convertases 1 and 2 (PC1 and PC2), carboxypeptidase E (CPE) and peptidyl α -amidating mono-oxygenase (PAM). PC1 is responsible for the first step in which the 32 kDa preprotein is cleaved to form proACTH and β -lipotrophin (β -LPH) (Figure 2). While β -LPH will be further processed to form β -MSH and β -endorphin, ProACTH will be further cleaved by PC1 to generate ACTH 1-39. PC2 will then cleave ACTH 1-39 to generate ACTH1–17 [for review see 173]. The generation of mature α -MSH from ACTH1–17 is catalyzed by carboxypeptidase E (CPE), peptidyl α -amidating mono-oxygenase (PAM) and a n-acetyltransferase. First, carboxy-terminal basic amino acids are trimmed from ACTH1–17 by CPE (Fig. 2). The peptide is subsequently amidated by PAM to generate desacetyl α -MSH (Des α -MSH). This is converted to mature α -MSH by a n-acetyltransferase that has not yet been identified. CPE and PAM are also believed to have additional functions in peptide processing. CPE may play a role in targeting prohormones into secretory granules [36] while PAM may interact with components of the actin cytoskeleton and thus may influence the trafficking of secretory granules [35]. Thus, the generation of α -MSH in the hypothalamus requires a complex pathway of posttranslational modification, involving numerous enzymes. It is therefore possible that alterations in any step of the POMC posttranslational modifications may have effects on energy metabolism. Impaired POMC processing has indeed been reported to be responsible for mouse models of obesity [154] and human obesity [104]. For example, the *Cpe* fat mouse displays a recessive obesity syndrome which is associated with high circulating levels of prohormones [154] due to the inactivation of carboxypeptidase E. In humans, an obesity phenotype has been reported which is associated with mutations in the human prohormone convertase 1 gene [104] and increased levels of unprocessed POMC. Studies on the regulation of these enzymes have shown for example that leptin regulates PC2 *in vivo* [178]. In addition, PC1 and PC2 were found to be differentially regulated in the brain of *Cpe* fat mice [9] and *in vitro* data were provided suggesting that the biosynthesis and expression of PC1 and PC2 are regulated by leptin [158]. Furthermore, additional studies have reported that PC1 and PC2 are also regulated by other metabolic hormones such as glucocorticoids and thyroid hormones [56, 123, 124].

Thus, impaired processing of POMC in the central nervous system by altering the melanocortin signaling, may ultimately result in obesity.

Despite the well described anorectic function of α -MSH [101, 186], peripheral administration of α -MSH has not been reported to reduce food intake. In fact, Des- α -MSH and Act- α -MSH have been shown to be potent anorexigenic peptides only when administered centrally. Additionally, Act- α -MSH seems to have a greater ability to decrease food intake than Des- α -MSH probably due to its greater stability. Nevertheless, these data strongly suggest that both Act- α -MSH and Des- α -MSH are rapidly degraded. Furthermore,

the degradation of α -MSH is independent of the presence of MC4R, although it has been reported that MC4R undergoes an internalization process that is dependent on its binding to α -MSH [195].

5. Prolyl carboxypeptidase

New data have recently suggested that prolyl carboxypeptidase (PRCP) is the enzyme responsible for the degradation of α -MSH [216]. Evidence was provided that hypothalamic PRCP is in an anatomical position to determine the efficacy of released α -MSH, thereby controlling the output and thus the efficacy of the melanocortin system (Fig. 3). In support of this idea, mice lacking PRCP display a lean phenotype when exposed to either a regular diet or high fat diet. Furthermore, in support of its function, PRCP has been previously associated with metabolic syndrome in human males [135].

5.1 Enzymatic properties

Prolyl carboxypeptidase (PRCP) is an enzyme of the family of the carboxypeptidase (CPs) which contains a serine residue in the active center, essential for catalytic activity that reacts with organophosphorus inhibitors. PRCP cleaves only peptides with a penultimate proline residue.

PRCP was first discovered in 1968 during studies of bradykinin metabolism in kidney [225]. It was found that after removal of the C-terminal Arg of bradykinin, the Pro-Phe-OH bond, which is usually resistant to carboxypeptidases, was cleaved by a kidney extract [225]. Since angiotensin II has the same C-terminus, it was tested and found to be a substrate of the enzyme. The enzyme was initially named angiotensinase C but later it was shown that the enzyme cleaves a variety of Pro-X bonds, thus, it was renamed PRCP.

PRCP is a single chain protein of approximately 58 kDa [225, 226]. The cDNA for human PRCP was cloned from a human kidney library [206] and based on that, the molecular weight of the mature protein should be around 51 kDa, indicating the presence of about 12% carbohydrate by weight [206]. This is consistent with the existence of six potential N-linked glycosylation sites. The deduced protein sequence contains 496 amino acids and includes a 30 residue signal peptide and a 15 amino acid propeptide [206]. The C-terminal half of the sequence contains an interesting "serine repeat" in which Ser is repeated as the 26th residue 6 out of 9 times [206]. In the other three repeats, Ser is replaced by Thr, Gly or Glu. When the Ser residues are aligned to yield 26 residue repeats, the positions of some of the other residues also fall into a pattern. The significance of this repeat region is not clear, but it might be involved in the maintenance of a secondary or tertiary structural motif or in the formation of the homodimer. The gene encoding human PRCP is on chromosome 11, while in the mouse it is on chromosome 7.

PRCP is inhibited by compounds that react with the active site serine residue. However, this action is not true for all serine protease inhibitors, such as aprotinin, or by chelating agents or inhibitors that react specifically with the active site cysteine in sulfhydryl proteases. Particularly, PRCP is not inhibited by non-specific SH-reactive agents, but is relatively

sensitive to the prolylendopeptidase inhibitor, Cbz-Pro-prolinal, because of its specificity for peptides with penultimate proline residues [161, 206].

5.2 Function

The functions of PRCP in regulating peptide actions in vivo have not been explored in detail. As a carboxypeptidase, PRCP catalyzes only the hydrolysis of the C terminal peptide bond, releasing one amino acid at a time. Although the cleavage of one amino acid from the C-terminus of a peptide or protein could appear to be of limited importance, it often results in profoundly altered physiological activity of such molecules [199, 216].

Specifically, PRCP cleaves peptides only if the penultimate residue is proline. Of the short synthetic substrates, Cbz-Pro-Ala is hydrolyzed fastest [161]. The enzyme does not cleave the Pro-Pro-COOH or (OH)-Pro-Pro-COOH bond [161]. Because the enzyme retains significant activity at neutral pH with naturally occurring substrates, such as angiotensin [102, 161], PRCP may also be active extracellularly.

The known substrates for PRCP are angiotensin II and III, prekallikrein [225, 226] and most recently α -MSH [216].

PRCP has an acidic pH optima (≈ 5.0) when hydrolyzing short synthetic peptide substrates [102, 161, 206], but it has been found that it retains significant activity at neutral pH range. Indeed, at physiological pH, α -MSH has been shown to be a better substrate than the well studied angiotensin II [216]. The enzymatic degradation of α -MSH₁₋₁₃ by PRCP results in the formation of α -MSH₁₋₁₂. When centrally administered, α -MSH₁₋₁₂, was found to be inactive in regulating food intake and also unable to induce increases in the frequency of action potentials of MC4R neurons of the hypothalamic paraventricular nucleus [216]. In addition, central and peripheral inhibition of PRCP enzymatic activity by inhibitors showed a significant reduction in food intake [216]. In addition, PRCP ablated (*PRCP^{gt/gt}*) mice are more sensitive to peripheral administration of α -MSH compared to wild type controls and, *PRCP^{gt/gt}* mice are leaner than controls on a regular and high-fat diet [216].

Besides α -MSH, PRCP could also regulate other peptides with a proline as the penultimate amino acid, the function of which is associated with body weight regulation. A few other hypothalamic peptides have a penultimate proline and they include Neuropeptide-glutamic acid-isoleucine (NEI; P56942; EIGDEENSAKFPI), Apelin 13 (Q9R0R4; QRPRLSHKGPMPF) and ghrelin (Q9EQX0; GSSFLSPEHQKAQRKESKKPPAKLQPR).

NEI, a peptide originating from the pro-MCH 131–164 region, has been found to be stronger than MCH in stimulating rat feeding behavior [134]. Thus, its effect on food intake would not be in line with the phenotype that was observed in the PRCP-ablated mouse. The same conclusion is true for ghrelin, the most potent circulating orexigenic hormone.

However, conflicting data have been reported on Apelin 13 [15, 164, 204, 207, 215]. Thus, future studies are needed to test whether Apelin 13 is a substrate for PRCP.

Finally, one cannot exclude the possibility that other peptides with a proline as the penultimate amino acid, the function of which is still unknown or not shown to be related to metabolism regulation, may be the cause of the PRCP-ablated mouse phenotype.

5.3 Localization

PRCP activity has been detected in a variety of cells and organs [102, 119, 198]. Within the cells, PRCP activity has been found in lysosomes and the cellular membrane [119, 131, 198–200]. PRCP has also been found to be released in response to stimulation and appears in the extracellular medium or biological fluids [141, 225]. In addition, lysosomal enzymes can associate with plasma membranes after exocytosis, bound to other transmembrane or membrane-associated proteins [200]. In addition, this serine carboxypeptidase can block part of the signal transduction stimulated by peptides. PRCP cleaves peptides after ligand-mediated receptor endocytosis [67, 150]. This usually involves fusion of the endosomes with lysosomes and results in peptide degradation and receptor recycling [226]. This process could be especially important in the metabolism of peptides that otherwise have a long-lasting effect.

PRCP has been found to be widely distributed, and its mRNA expression is highest in human placenta, lung and liver [206]. PRCP is also present in white blood cells and fibroblasts, and is highly expressed in endothelial cells [119, 200]. In mice, like humans, PRCP is expressed in peripheral tissues such as kidney, liver, heart and spleen. Centrally, PRCP is expressed in the hypothalamus and brainstem. Within the hypothalamus, higher expression of PRCP was detected in the perifornical-lateral hypothalamic region and dorsomedial nucleus [216]. This anatomical data suggest that hypothalamic PRCP is in an ideal position to determine the efficacy of released α -MSH, thereby controlling the output of the melanocortin system. PRCP is mainly expressed in the DMH where MC4R-expressing neurons are localized and in the lateral hypothalamic hypocretin/orexin [Hcrt] and melanin-concentrating hormone (MCH) neurons. A relatively small number of MC4R-expressing neurons have been found in the LH, including the perifornical area [127]. However, Hcrt and MCH neurons project to various areas of the hypothalamus, including the PVN, where α -MSH terminals strongly innervate MC4R-expressing neurons.

Thus, PRCP could be released in the PVN from the Hcrt and MCH axon terminals into the synaptic space where it would then degrade α -MSH extracellularly (Fig. 3). However, high expression of MC4R-containing neurons has been shown in the hypothalamic DMH [127] where abundant expression of PRCP was detected and where arcuate α -MSH-containing fibers project [197]. Thus, in the DMH, PRCP could be involved in the intracellular peptide degradation and receptor recycling (Figure 3). In conclusion, PRCP may act in different hypothalamic sites to modulate melanocortin efficacy. Once released from the Hcrt and/or MCH terminals in the PVN, PRCP will degrade α -MSH_{1–13} extracellularly (Fig. 3) thus increasing the antagonist effect of AgRP, while in the DMH, PRCP will degrade α -MSH intracellularly thus shortening the effect of α -MSH on MC4R and enhancing the overall orexigenic tone of the melanocortin system (Fig. 3).

5.4 A potential role for PRCP in leptin resistance

A postulated cause for the development of obesity is leptin resistance. As fat stores increase during the development of obesity, circulating leptin levels are continuously rising. However, these elevated leptin levels or administration of exogenous leptin to obese subjects does not lead to decreased body weight. This discrepancy between leptin levels and the metabolic phenotype is attributed to leptin resistance [152]. A primary site of leptin resistance is suggested to be the POMC neurons [152]. Indeed, intracellular signaling cascades triggered by leptin receptor activation were found to be impaired in diet-induced obese animals [12, 151]. Specifically, activation of the transcription factor STAT3 by leptin is diminished in these animals [12, 151], an event that is attributed to the inhibitory action of SOCS3 on STAT3 phosphorylation [12]. Additionally, *ex vivo* studies revealed that leptin induced secretion of α -MSH₁₋₁₃ is reduced from hypothalamic slices taken from diet-induced obese mice [66]. An alternative explanation for the development of leptin resistance was the decreased ability of leptin from the circulation to reach hypothalamic neurons [27]. Despite of all these elegant and logical data, several parameters of POMC neurons in diet induced obesity show alterations completely consistent with elevated leptin levels. For example, the wiring of the melanocortin system and transcript levels of POMC and AgRP/NPY of obese animals in response to a hypercaloric environment reflects an adequate and possibly maximal responsiveness of these cells to leptin [100]. The synaptic organization triggered in these animals shows similar trends of changes to those that were described previously for highly leptin sensitive *ob/ob* mice in response to leptin administration [170]. Although reactive gliosis was also detected in response to high fat diet specifically associated with arcuate nucleus capillaries [100], this gliosis does not appear to block the effect of leptin on the synaptic input organization of the POMC neurons and POMC, NPY and AgRP transcript levels, and hence, may not be the cause of leptin resistance as proposed earlier [27]. On the other hand, it is conceivable that assays using hypothalamic slices to measure α -MSH release [66] may be skewed by gliosis, an event that could be a structural obstacle for the release of measurable α -MSH into the medium, thereby limiting the usefulness of these assays for the study of α -MSH release kinetics. Thus, if neurobiological attributes of leptin action are present in the hypothalamus of diet-induced obese animals, it is a reasonable suggestion that the degradation of released α -MSH₁₋₁₃ may interfere with the ability of MC4R activation in POMC target sites. As described above, PRCP location and function in the hypothalamus makes this carboxypeptidase, although previously unsuspected, a player in the emergence of systemic leptin resistance as it relates to the regulation of food intake, energy expenditure and glucose homeostasis.

Concluding remarks

Many hormonal and nutritional signals have been shown to directly affect hypothalamic melanocortin signaling by either activating or inhibiting transcriptional, translational and post-translational POMC processes. In addition, many of the signaling pathways activated by these circulating signals have been found to act via common denominators. Most recently, a new component of the melanocortin signaling, PRCP, has been revealed.

Although it was discovered over 40 years ago, limited studies on PRCP function and regulation have been performed. Some functions are related to its specificity to cleave after proline residues, restricting its substrates to fewer peptides. In the hypothalamus, α -MSH has been recently shown to be a substrate for PRCP [216]. Thus, due to its involvement in α -MSH degradation, it is possible that PRCP is an important component of the melanocortin signaling downstream of the melanocortin neurons thereby determining the efficacy of the melanocortin signaling. Thus, an increase in the activity of PRCP as a result of a decrease in α -MSH levels in the hypothalamus, may promote metabolic dysfunctions, including obesity and type 2 diabetes. Therefore, future studies on the role, function and regulation of PRCP under different metabolic conditions are needed and may reveal it to be a potential drug target for such metabolic disorders.

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The central melanocortin system plays a critical role in the regulation of metabolism.

α -melanocyte stimulating hormone (α -MSH) is the most potent anorectic peptide.

Many circulating signals regulate α -MSH production and activity.

The enzymatic inactivation of α -MSH plays an important role in metabolism

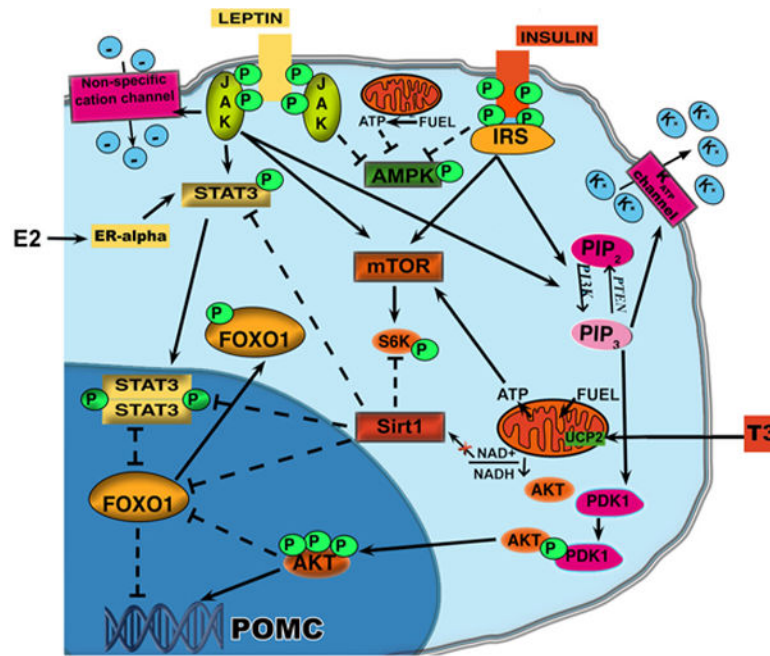


Figure 1.

Schematic illustration showing the metabolic pathways activated by hormones and fuel availability in POMC neurons of the arcuate nucleus of the hypothalamus. Activation of PI3K in POMC neurons by either leptin or insulin receptors induces activation of AKT which leads to the phosphorylation of FoxO1 and thus its exclusion from the nucleus allowing the transcription of POMC. Concomitantly, PI3K activation also induces the opening of fewer potassium ATP channels thus inducing the leptin-dependent firing of the POMC neurons and leptin-dependent POMC transcription via Stat3. Stat3 has been also reported to be a target of estrogen signaling. However, the effect of estrogen on Stat3 is JAK independent. In satiety conditions when POMC neurons are activated, Sirt1 and NAD⁺ levels are decreased and Sirt1 activation of FoxO1 is also inhibited. Finally, the decrease in the ratio AMP/ATP will decrease AMPK while increase mTOR activity.

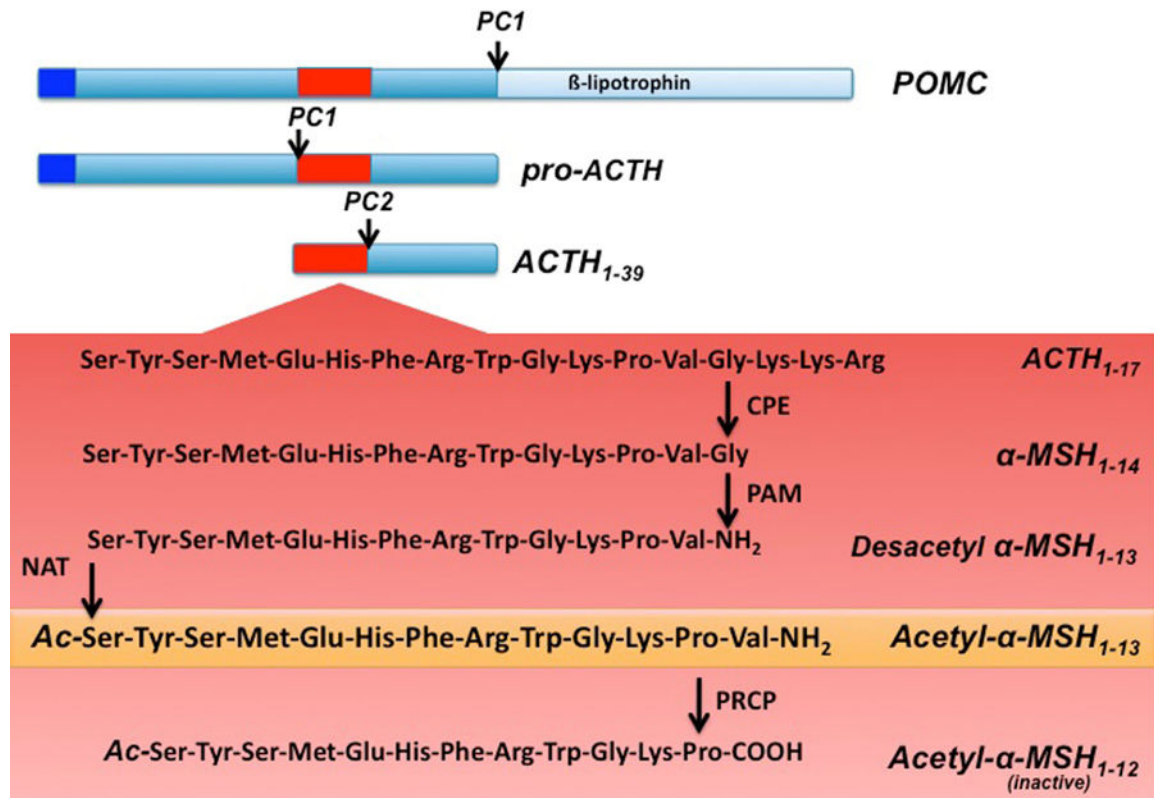


Figure 2. Schematic illustration of hypothalamic POMC processing. Prohormone convertase 1 (PC1) cleaves the 32 kDa preprotein into proACTH and β -lipotrophin . ProACTH is then further converted by PC1 to form ACTH₁₋₃₉. Prohormone convertase 2 (PC2) will then cleave ACTH₁₋₃₉, to form ACTH₁₋₁₇. ACTH₁₋₁₇ will be first processed by carboxypeptidase E (CPE) and then α -amidating mono-oxygenase (PAM) to form desacetyl α -MSH₁₋₁₃. An n-actetyltransferase (NAT) will then convert desacetyl- α -MSH₁₋₁₃ into the more active form acetyl- α -MSH₁₋₁₃. Finally acetyl- α -MSH₁₋₁₃ will be degraded by prolyl carboxypeptidase (PRCP) into the inactive product acetyl- α -MSH₁₋₁₂.

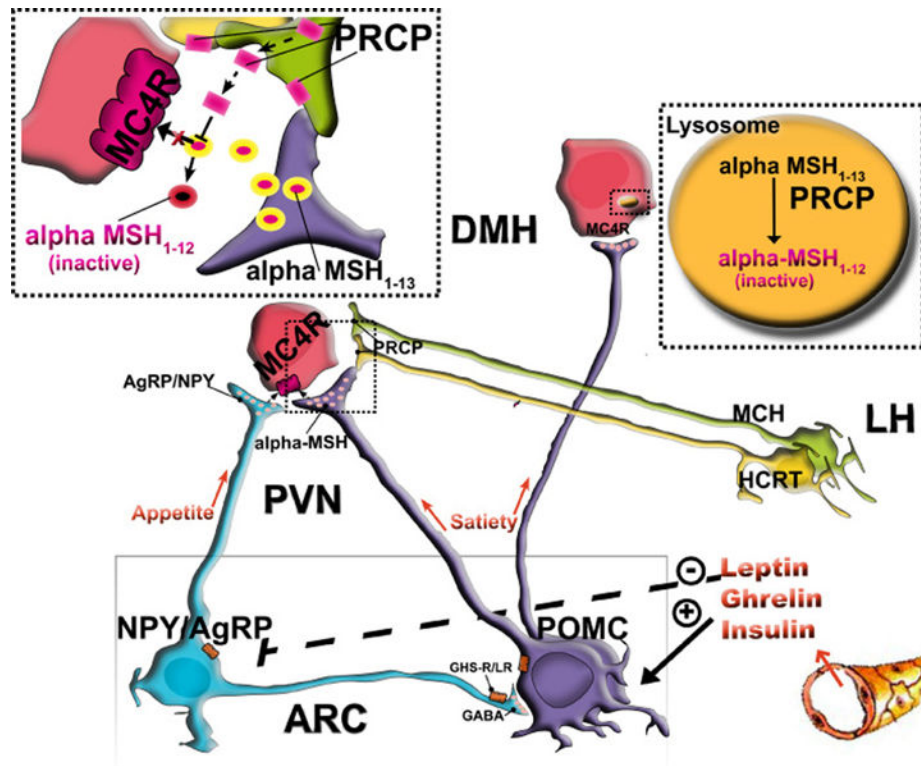


Figure 3.

PRCP is in an anatomical position to determine the efficacy of released α -MSH, thus controlling the output of the melanocortin system. PRCP is mainly expressed in the lateral hypothalamus (LH) in hypocretin/orexin (Hcrt) and melanin-concentrating hormone (MCH) neurons and in the hypothalamic dorsomedial nucleus (DMH) where MC4R-expressing neurons are located. The Hcrt and MCH neurons project to various areas of the hypothalamus, such as the paraventricular nucleus, where α -MSH terminals strongly innervate MC4R-expressing neurons. When released from the Hcrt and/or MCH terminals, PRCP will degrade α -MSH extracellularly, thus increasing the antagonistic effect of AgRP. In the DMH, however, PRCP degrades α -MSH intracellularly, thereby enhancing the overall orexigenic tone of the system.