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Reduced levels of neurotransmitter-degrading enzyme PRCP promote obesity

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Commentary

The level of neurotransmitters present in the synaptic cleft is a function of the delicate balance among neurotransmitter synthesis, recycling, and degradation. While much is known about the processes controlling neurotransmitter synthesis and release, the enzymes that degrade peptide neurotransmitters are poorly understood. A new study in this issue of the *JCI* reveals the important role of neuropeptide degradation in regulating obesity (see the related article beginning on page 2291). Wallingford et al. provide evidence that, in mice, the enzyme prolylcarboxypeptidase (PRCP) degrades α -melanocyte-stimulating hormone (α -MSH) to an inactive form that is unable to inhibit food intake. Their studies indicate that PRCP expression promotes obesity, while inhibitors of the enzyme counteract obesity.

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Reduced levels of neurotransmitter-degrading enzyme PRCP promote obesity

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The level of neurotransmitters present in the synaptic cleft is a function of the delicate balance among neurotransmitter synthesis, recycling, and degradation. While much is known about the processes controlling neurotransmitter synthesis and release, the enzymes that degrade peptide neurotransmitters are poorly understood. A new study in this issue of the *JCI* reveals the important role of neuropeptide degradation in regulating obesity (see the related article beginning on page 2291). Wallingford et al. provide evidence that, in mice, the enzyme prolylcarboxypeptidase (PRCP) degrades α -melanocyte-stimulating hormone (α -MSH) to an inactive form that is unable to inhibit food intake. Their studies indicate that PRCP expression promotes obesity, while inhibitors of the enzyme counteract obesity.

Neurons in the arcuate region of the hypothalamus that make the polypeptide hormone precursor proopiomelanocortin (POMC) are very important for body weight regulation, as revealed by the dramatic development of obesity after selective

inactivation of the neuronal *Pomc* gene in mice (1) and the presence of *POMC* mutations in humans with severe early-onset obesity (2). POMC is proteolytically processed into a number of physiologically important peptides, including endorphins, adrenocorticotrophic hormone (ACTH), and α -melanocyte-stimulating hormone (α -MSH). The loss of α -MSH production by POMC-deficient neurons is primarily responsible for the resultant obesity (3). The 13-amino-acid peptide α -MSH (referred to as α -MSH₁₋₁₃) acts on the postsynaptic melanocortin 4 receptor (MC4R) in many brain

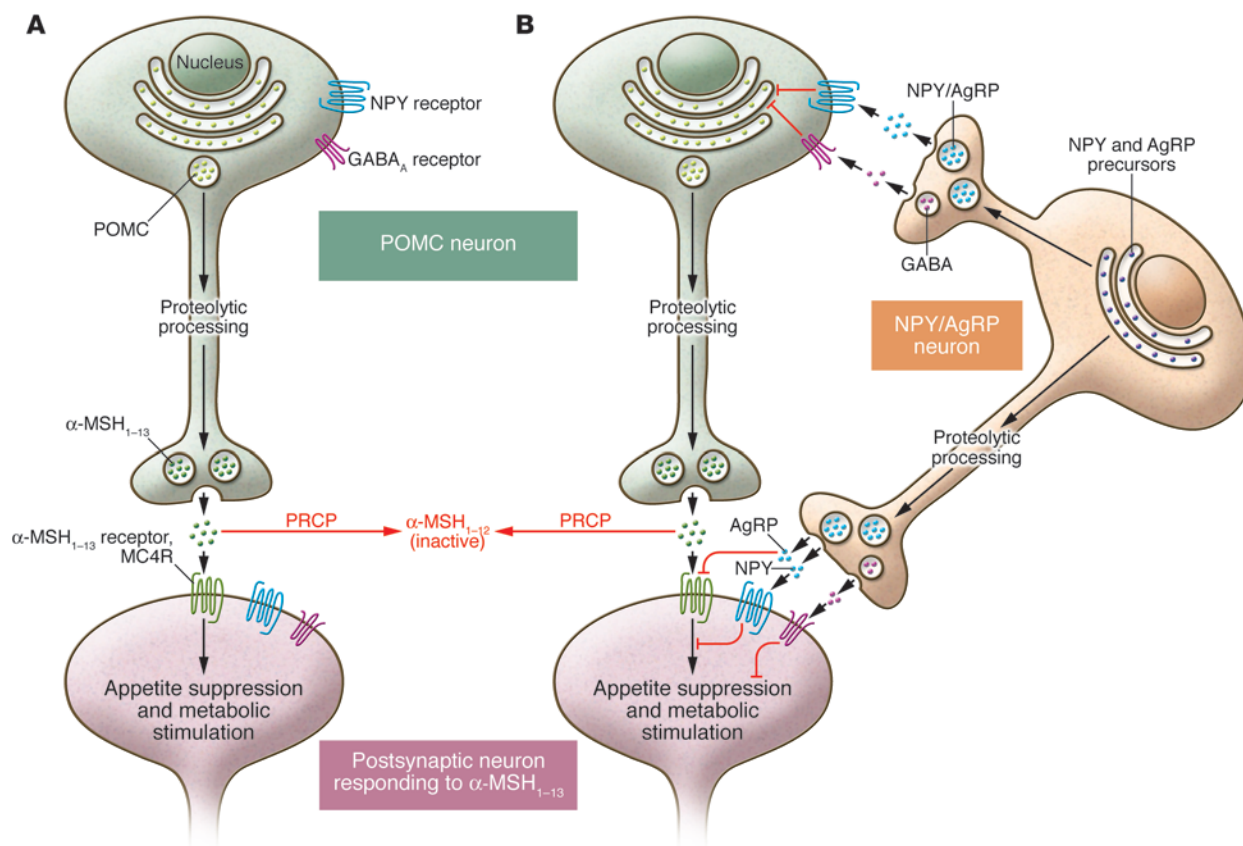
regions to suppress appetite and stimulate metabolism (Figure 1). Failure of α -MSH₁₋₁₃ signaling due to mutations in the *MC4R* gene represents the most common form of genetically inherited human obesity (4).

The activity of POMC neurons and the synthesis of POMC are stimulated by hormones such as leptin and insulin and inhibited by fasting (5–7). Signaling by POMC neurons is kept in check by neighboring neurons that make neuropeptide Y (NPY), agouti-related protein (AgRP), and GABA (Figure 1). The hormones and neurotransmitters that regulate these two populations of hypothalamic neurons have been studied extensively during the last 15 years (5–7). Whereas the events that lead up to activation of MC4R by α -MSH₁₋₁₃ are well known, virtually nothing is known about how α -MSH₁₋₁₃ activity is terminated. The extent of MC4R activation, and hence the extent of appetite loss, will be determined by the rate of α -MSH₁₋₁₃ release from POMC neurons and the rate at which it is degraded or otherwise cleared from the synaptic space. A seminal paper

Conflict of interest: The author has declared that no conflict of interest exists.

Nonstandard abbreviations used: ACTH, adrenocorticotrophic hormone; MC4R, melanocortin 4 receptor; α -MSH, α -melanocyte-stimulating hormone; POMC, proopiomelanocortin; PRCP, prolylcarboxypeptidase.

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**Figure 1**

Neural circuitry in the hypothalamus that regulates feeding and metabolism. **(A)** The POMC pathway. Activation of neurons that make POMC inhibits feeding and stimulates metabolism. Under conditions of energy excess, POMC neurons are activated by circulating hormones and neurotransmitters. POMC neurons process the POMC precursor protein to α -MSH₁₋₁₃ within the secretory pathway, as shown in Figure 2. When these neurons are activated, they release α -MSH₁₋₁₃ into the synaptic cleft, where it can bind to melanocortin receptors (MC4R) on postsynaptic cells located in several brain regions. Mutations in genes responsible for making α -MSH or receiving the α -MSH signal result in obesity. The action of α -MSH₁₋₁₃ is terminated by the action of PRCP (the present study, ref. 8), which is presumably released from cells in the postsynaptic region; however, the identity of the cells that make PRCP and its cellular/extracellular localization are not currently known. The activity of PRCP thus puts a brake on α -MSH signaling; mice that lack PRCP are lean because they have excessive α -MSH signaling. **(B)** Activation of neuropeptide Y/agouti-related protein (NPY/AgRP) neurons counteracts the activity of POMC neurons. When energy balance is low, e.g., during starvation, signaling by the expression of POMC is reduced and the activity of POMC neurons is inhibited by the neighboring NPY/AgRP neurons that become activated and release NPY, AgRP, and GABA. NPY and AgRP are also neuropeptides that are processed from larger precursor proteins. NPY acts on G protein-coupled receptors that couple to *G_{ai}* and hence counter the action of α -MSH₁₋₁₃, which acts on *G_{as}*-linked receptors; AgRP binds to the MC4R and prevents α -MSH signaling; and GABA, acting on ionotropic GABA_A receptors, hyperpolarizes the POMC and postsynaptic neurons, thereby reducing their activity.

by Wallingford et al., reported in this issue of the *JCI*, provides the first insights into how α -MSH₁₋₁₃ is inactivated (8).

Proteolytic processing of POMC

A brief overview of how α -MSH₁₋₁₃ is created from POMC (4, 9) facilitates appreciation of the mechanism of its inactivation reported by these authors (Figure 2). POMC is cleaved at dibasic residues by proconvertase 1 (PC1), resulting in the release of ACTH₁₋₃₉ from the middle of the POMC precursor molecule. ACTH₁₋₃₉ is further cleaved by PC2 at a tetrabasic sequence to

generate ACTH₁₋₁₇. The basic residues at the C terminus (Lys-Lys-Arg) are removed by carboxypeptidase E, and the newly exposed C-terminal glycine is converted to an amide by peptidyl α -amidating monooxygenase (PAM) to yield desacetyl- α -MSH₁₋₁₃, which is then acetylated on the N-terminal serine residue, resulting in the formation of mature, active α -MSH₁₋₁₃. All of these processing events occur during transit of the POMC precursor through the regulated secretory pathway in hypothalamic neurons so that vesicles containing mature, active α -MSH₁₋₁₃ can be released

when POMC neurons are activated. Deficits in these processing enzymes also lead to obesity, at least in part because of inadequate α -MSH₁₋₁₃ production (10–12).

Wallingford et al. (8) now show that α -MSH₁₋₁₃ is inactivated by removal of the amidated C-terminal valine residue. The enzyme involved in this inactivation is prolylcarboxypeptidase (PRCP) (Figure 2). This serine protease was discovered more than 40 years ago because of its ability to convert the vasoconstrictor angiotensin II to angiotensin₁₋₇, which counteracts vasoconstriction (13). Later, PRCP was shown

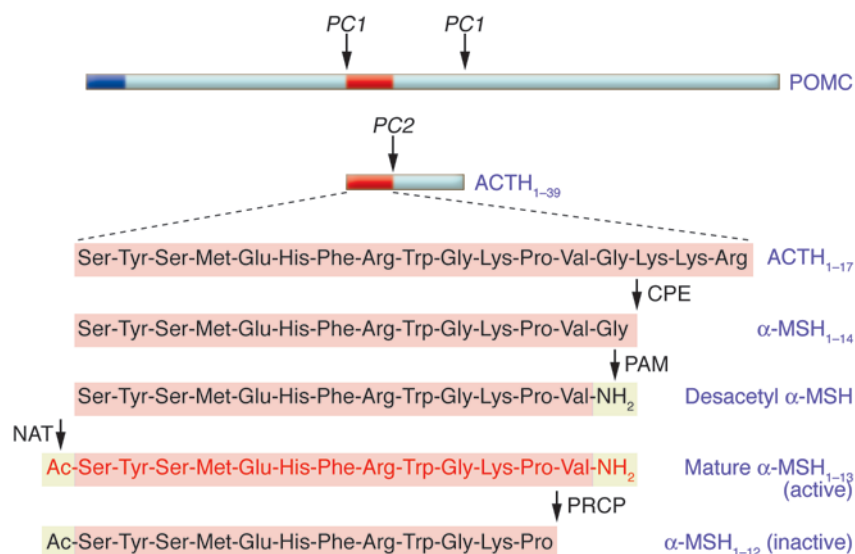


Figure 2

Processing of POMC to α-MSH. POMC is synthesized in brain, pituitary gland, and skin cells. It is transported into the secretory pathway and processed by several enzymes into a variety of active peptides, depending on the cells in which it is synthesized. In hypothalamic neurons, POMC processing to produce α-MSH is particularly important. The POMC precursor is first cut by proconvertase 1 (PC1) to release ACTH₁₋₃₉, which is then cut by proconvertase 2 (PC2) to produce α-MSH₁₋₁₇. Carboxypeptidase E (CPE) removes the basic amino acids (Lys-Lys-Arg) from the C terminus, and peptidyl α-amidating monooxygenase (PAM) converts the C-terminal glycine to an amide (NH₂). The N terminus of α-MSH₁₋₁₃ is acetylated by N-acetyltransferase (NAT) to produce mature, functional α-MSH₁₋₁₃. After release from synaptic vesicles, α-MSH₁₋₁₃ is inactivated (to α-MSH₁₋₁₂) by the action of PRCP, the subject of the Wallingford et al. (8) study in this issue of the *JCI*.

to activate the coagulation-related protein prekallikrein – an early step in the production of the vasodilator bradykinin (14). The current study by Wallingford et al. (8) shows that this enzyme is also expressed in the brain, where it plays an important role in determining neuropeptide levels. Their studies began with a C57BL/6 mouse strain that carries a small region of chromosome 7 from a BALB/c strain. Those mice were significantly leaner compared with C57BL/6 mice without the BALB/c chromosomal segment (15), prompting further investigation as to the gene(s) in that chromosomal interval that contribute to the lean phenotype. Further studies determined that the relevant BALB/c region on chromosome 7 includes only 4 protein-encoding genes; of those genes, *Prcp* was the likely candidate, given previous reports of a link between PRCP and metabolic syndrome (16). Sequencing revealed no polymorphisms within any of the coding regions of the 4 genes, but there was a single-base polymorphism at -718 in the presumptive promoter/enhancer region of the *Prcp* gene that was associated with

a significant reduction in the level of *Prcp* mRNA in the brain of the mice carrying the BALB/c allele. The authors also generated a complete knockout of the *Prcp* gene from mouse embryonic stem cells in which a *lacZ* gene had randomly inserted into the fourth intron of the *Prcp* gene in a gene-trapping experiment. The *Prcp*-null (*Prcp*^{-/-}) mice ate less and had even less fat than the mice with partial loss of the enzyme; furthermore, these animals were resistant to diet-induced obesity. The authors convincingly demonstrate that α-MSH₁₋₁₃ is a substrate for PRCP, which is known to remove the carboxyterminal residue from proteins with a penultimate proline residue. As predicted, the *Prcp*^{-/-} mice had elevated levels of α-MSH₁₋₁₃ in the brain. Injection of α-MSH₁₋₁₃ into the brain of wild-type mice inhibited feeding, whereas injection of the same amount of the truncated, inactive α-MSH₁₋₁₂ was ineffective at reducing food consumption. As a final demonstration of the importance of PRCP, the authors showed that delivery of 2 different specific inhibitors of PRCP to the brain could suppress food intake by hungry mice in an

MC4R-dependent manner. Overall, this is a very satisfying set of results that logically leads from the identification of a candidate disease gene to determination of its mechanism of action.

PRCP is expressed in the hypothalamus

Wallingford et al. (8) show that many cells in the brain make PRCP, including cells in the hypothalamus, where α-MSH₁₋₁₃ is released onto postsynaptic neurons bearing MC4 receptors. PRCP has a signal peptide to direct it into the secretory pathway. Although PRCP was originally described as a lysosomal enzyme (17), it seems likely that its action on its known substrates, including α-MSH₁₋₁₃, occurs extracellularly. So, PRCP may be poised in the synaptic space to degrade α-MSH₁₋₁₃ and thereby reduce α-MSH₁₋₁₃ action, which would promote anorexia. It is also possible that the amount of PRCP in the extracellular space can be regulated; e.g., under starvation conditions, the activity of PRCP may be elevated in order to limit α-MSH₁₋₁₃ signaling and thereby help promote feeding and reduce metabolism. The converse situation has been demonstrated by studies showing that acetylation of desacetyl α-MSH₁₋₁₃ increases in response to the adiposity hormone leptin (17, 18), which stabilizes α-MSH₁₋₁₃ and thereby promotes weight loss.

Future directions

The present study adds an important new dimension to our understanding of how neuropeptide signaling is controlled in the brain. Moreover, it raises many intriguing questions and possibilities. Considering that α-MSH₁₋₁₃ also functions in the skin to control pigmentation via activation of melanocortin-1 receptors, changes in PRCP levels may also affect skin and hair color. Because PRCP is widely expressed at low levels in the brain and in the periphery, it is likely that there are additional substrates with penultimate proline residues in their sequence whose levels are controlled by PRCP. Consequently, the phenotype of the *Prcp*^{-/-} mice may be more complex, and more interesting, than currently envisioned. For example, we can already anticipate that the levels of angiotensin II and bradykinin will be affected. Thus, there is much more to learn from the *Prcp*^{-/-} mice generated by Wallingford and colleagues (8). It will also be interesting to determine whether mutations in the human *PRCP*



gene are correlated with body weight. If it turns out that α -MSH₁₋₁₃ is the principal substrate of PRCP, then pharmacological inhibition of its action in the brain could help reduce appetite and weight gain; Wallingford et al. (8) demonstrated the efficacy of this strategy by injecting inhibitors directly into the brain, but brain-penetrating drugs would need to be developed to be clinically useful.

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Engaging the lysosomal compartment to combat B cell malignancies

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The combination of rituximab, a type I anti-CD20 mAb, with conventional chemotherapy has significantly improved the outcome of patients with B cell malignancies. Regardless of this success, many patients still relapse with therapy-resistant disease, highlighting the need for the development of mAbs with higher capacity to induce programmed cell death. The so-called type II anti-CD20 mAbs (e.g., tositumomab) that trigger caspase-independent B cell lymphoma cell death in vitro and show superior efficacy as compared with rituximab in eradicating target cells in mouse models are emerging as the next generation of therapeutic anti-CD20 mAbs. In this issue of the JCI, Ivanov and colleagues identify the lysosomal compartment as a target for type II mAbs (see the related article beginning on page 2143). These data encourage the further clinical development of type II mAbs as well as other lysosome-targeting drugs in the treatment of B cell malignancies.

Caspase-mediated apoptosis is the main mechanism of action of most current anti-cancer treatments. Defects in apoptosis signaling pathways are, however, among the

major hallmarks of cancer, and apoptosis-inducing therapies further select for highly apoptosis-refractory tumor cell clones (1). Accordingly, new strategies to kill cancer cells by nonapoptotic mechanisms have flourished during the past decade, and many mediators of alternate cell death pathways have been identified. Among them are the lysosomes with their large arsenal of proteolytic and lipolytic hydrolases (2, 3).

The lysosome: an emerging target for cancer therapy

Lysosomes function as cellular recycling and waste disposal units by degrading organelles and macromolecules delivered to the lysosomal compartment by autophagy, endocytosis, and phagocytosis (4). The degradation is performed by over 50 lysosomal hydrolases that can process all the major macromolecules of the cell to break down products available for metabolic reutilization. The lysosomal cell death pathways are characterized by partial lysosomal membrane permeabilization and the subsequent translocation of lysosomal hydrolases into the cytosol (2, 3). Once in the cytosol, lysosomal hydrolases, particularly cathepsin proteases, can trigger caspase-independent and BCL-2-insensitive cell death pathways even in highly apoptosis-resistant cancer cells.

The potential of lysosomes as powerful “cell suicide bags” was recognized already in the 1950s by Christian de Duve, who received the Nobel prize for his discovery of

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Nonstandard abbreviations used: Ag, antigen.

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