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Anatomy and regulation of the central melanocortin system

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The central melanocortin system is perhaps the best-characterized neuronal pathway involved in the regulation of energy homeostasis. This collection of circuits is unique in having the capability of sensing signals from a staggering array of hormones, nutrients and afferent neural inputs. It is likely to be involved in integrating long-term adipostatic signals from leptin and insulin, primarily received by the hypothalamus, with acute signals regulating hunger and satiety, primarily received by the brainstem. The system is also unique from a regulatory point of view in that it is composed of fibers expressing both agonists and antagonists of melanocortin receptors. Given that the central melanocortin system is an active target for development of drugs for the treatment of obesity, diabetes and cachexia, it is important to understand the system in its full complexity, including the likelihood that the system also regulates the cardiovascular and reproductive systems.

The gene encoding pro-opiomelanocortin (POMC) produces two different classes of peptides, melanocortins and β-endorphins, which have diverse functions as both hormones and neuropeptides. The melanocortin peptides, which include adrenocorticotropin and α-, β- and γ-melanocyte–stimulating hormones (MSH), mediate their effects through a family of five related G protein–coupled melanocortin receptors, MC1R through MC5R.

In the periphery, POMC is expressed primarily in the pituitary, skin and hair follicle. Adrenocorticotropin, derived from pituitary POMC, acts to regulate adrenal glucocorticoid production through the MC2R or adrenocorticotropin receptor. Melanocortin peptides in skin and hair act in a paracrine fashion to regulate the relative production of the yellow-red and brown-black pigments through the MC1R or MSH receptor expressed at these sites. MC5R is also expressed in the periphery in a variety of exocrine glands, where it seems to be involved in regulating the secretion of exocrine gland products.

In the brain, the mammalian central melanocortin system is defined as a collection of CNS circuits that include (i) neurons that express hypothalamic neuropeptide Y and agouti gene–related protein (NPY/AgRP) or POMC and that originate in the arcuate nucleus, (ii) brainstem POMC neurons originating in the commissural nucleus of the solitary tract (NTS) and (iii) downstream targets of these POMC and AgRP neurons expressing the melanocortin-3 (MC3R) and melanocortin-4 receptors (MC4R). In the CNS, melanocortin peptides are agonists of the MC3R and MC4R, whereas AgRP is a high-affinity antagonist of both these receptors. The melanocortin system is unique in that many melanocortin receptor sites in the CNS receive projections from both agonist-expressing POMC

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fibers and antagonist-expressing AgRP fibers, whereas some seem to receive only agonist innervation[1].

Another unique feature of the central melanocortin system is that it acts like a rheostat on energy storage[2]: there are gene dosage effects for the POMC and MC4R genes in which heterozygous loss of either gene produces intermediate phenotypes, a phenomenon unusual for G protein–coupled receptor signaling systems. In the mouse, obesity results from many different lesions to the system, including ectopic expression of the agouti protein, another endogenous antagonist of the MC4R, in the CNS (ref. 3), deletion of either MC4R (ref. 2) or MC3R (ref. 4), deletion of the POMC gene[5] or transgenic overexpression of AgRP[6].

The melanocortin obesity syndrome can occur in humans, as shown by an agouti mouse–like syndrome in two families, resulting from null mutations in the gene encoding POMC[7]. These patients have a rare syndrome that includes adrenocorticotropic hormone (ACTH) insufficiency, red hair and obesity, resulting from the lack of ACTH peptide in the serum, and a lack of α-MSH in skin and brain, respectively. These data demonstrate that the central melanocortin circuitry subserves energy homeostasis in humans, as it does in the mouse. Heterozygous frameshift mutations in the human *MC4R* gene, associated with non-syndromic obesity, were subsequently identified in two separate families[8,9]. Further studies now show that haploinsufficiency of the MC4R in humans is the most common monogenic cause of severe obesity, accounting for up to 5% of cases[10,11]. The detailed clinical picture of the syndrome[12,13] is virtually identical to that reported for the mouse[2,14,15], with increased adipose mass, increased linear growth and lean mass, hyperinsulinemia greater than that seen in matched obese controls and severe hyperphagia.

Although the severity of the obesity epidemic has focused attention on the need for better therapeutics and thus the role of the central melanocortin system in energy homeostasis, it is important to note that data also support a role of this system in cardiovascular and sexual function, as described in more detail below.

Anatomy of the hypothalamic melanocortin system

The major site of POMC expression in the CNS originates in neurons of the arcuate nucleus (**Fig. 1**). In mice there are approximately 3,000 such POMC-positive cells, most of which also express the anorectic peptide cocaine amphetamine–related transcript (CART). The POMC- and CART-positive (POMC/CART) cell bodies are found throughout the rostrocaudal extent of the arcuate nucleus and periarcuate area of the hypothalamus[16–18]. Proceeding from rostral to caudal, arcuate POMC cells send a dense bundle of fibers ventral to the anterior commissure, to several nuclei in the septal region, including the bed nucleus of the stria terminalis and lateral septal nucleus, as well as to the nucleus accumbens in the caudate putamen. More caudally, fibers are seen projecting to the periventricular region of the thalamus and to the medial amygdala. Within the hypothalamus, the densest fibers project to the periventricular nucleus, the paraventricular nucleus (PVH) and the periformical region, with some fibers seen in most hypothalamic regions. These hypothalamic POMC-expressing neurons (hereafter referred to as 'POMC neurons') also send two descending sets of projections to the brainstem: one via the periaquaductal gray and dorsomedial tegmentum, which is thought to innervate the rostral NTS and lateral reticular nucleus (A5–C1 cell groups), and another, believed to be the predominant descending bundle, via the ventral tegmental area, innervating the rostral NTS, ventrolateral medulla (A1 cell group) nucleus ambiguous and spinal cord. Approximately half of the α-MSH immunoreactivity in the brainstem is thought to derive from hypothalamic POMC neurons, and the other half derives from a smaller number (~300) of POMC neurons in the brainstem, described in more detail below[19,20].

Although the POMC neurons of the arcuate nucleus are defined neurochemically by virtue of their expression of POMC and CART peptides, controversy continues to exist regarding neurotransmitters that may also be present in these cells. GABAergic currents have been identified resulting from the autologous synapses formed by cultured POMC neurons purified on the basis of fluorescence from transgenic mice expressing green fluorescent protein (GFP) under the control of the POMC promoter[21]. Furthermore, approximately one-third of arcuate POMC neurons express the mRNA for glutamic acid decarboxylase. However, glutamate transporter (EAT3) immunoreactivity is also expressed in some of these cells, implying that some POMC neurons may be glutamatergic[22]. However, POMC neurons have not yet been shown to be functionally glutamatergic or GABAergic in a slice preparation.

As the arcuate NPY/AgRP-expressing neurons (hereafter referred to as 'NPY/AgRP neurons') express the potent MC3R and MC4R antagonist AgRP, they are also a critical component of the central melanocortin system. AgRP-immunoreactive fibers appear primarily in a subset of the same hypothalamic and septal brain regions containing dense POMC innervation, with the densest fibers found innervating the PVH, dorsomedial hypothalamus (DMH), posterior hypothalamus and septal regions around the anterior commissure[1,23]. Within the rat hypothalamus, POMC fibers have a much wider distribution than AgRP: moderate fiber density is seen in almost every nucleus, with the possible exceptions of the ventromedial hypothalamus (VMH) and supraoptic nucleus. In the limited number of studies published, AgRP-immunoreactive fibers were also notably absent in the rat in additional divisions of the neuraxis receiving POMC fibers, such as the rostral portion of the brain stem, hippocampus, amygdala, corpus striatum and olfactory cortex tract. Ultimately, an understanding of the nature of the MC3R- and MC4R-expressing target cells is also essential to completing this anatomical picture of the central melanocortin system, but space limitations preclude discussion of this topic here.
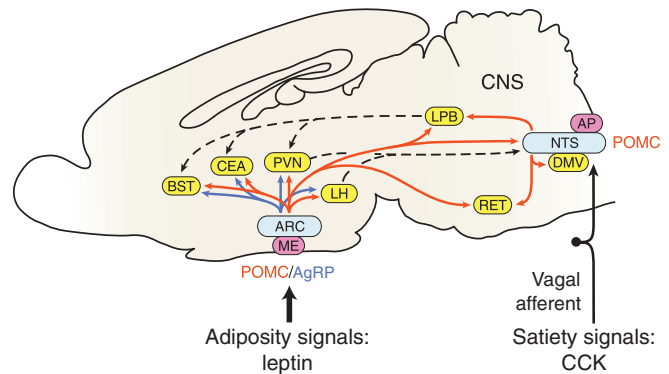
Figure 1 Schematic of the central melanocortin system. POMC neurons in the arcuate nucleus of the hypothalamus and the nucleus tractus solitarius of the brainstem are both adjacent to circumventricular organs and receive and integrate signals from adipostatic factors and satiety factors, respectively. Blue, nuclei containing POMC neurons; magenta, circumventricular organs adjacent to POMC neurons; yellow, a small sample of representative nuclei containing MC4R-positive neurons that may serve to integrate adipostatic and satiety signals; red arrows, representative POMC projections; blue arrows, representative AgRP projections; dashed arrows, secondary projections linking POMC neurons in hypothalamus and brainstem with common effector sites; AP, area postrema; ARC, arcuate nucleus; BST, bed nucleus of the stria terminalus; CEA, central nucleus of the amygdala; DMV, dorsal motor nucleus of the vagus; LH, lateral hypothalamic area; LPB, lateral parabrachial nucleus; ME, median eminence; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus of the hypothalamus; RET, reticular nucleus. Figure modified from ref. 92.

Hormonal regulation of the hypothalamic melanocortin system

Situated between the third ventricle and the median eminence, in which the portal vascular system functions to transport neuroendocrine releasing factors to the anterior pituitary, the arcuate nucleus is uniquely positioned to sample factors in both blood and cerebrospinal fluid (**Fig. 1**). Though not classically viewed as such, the arcuate nucleus may sometimes function as a circumventricular organ, existing functionally outside the blood-brain barrier. Although the mechanisms involved are not clear, hypophysiotropic neurons as well as arcuate POMC and NPY neurons have been shown to send fibers into the median eminence. The median eminence may thus contribute to the sensing and response by POMC/CART and NPY/AgRP neurons to blood-born hormone and nutrient signals relevant to both energy homeostasis and hunger/satiety signaling (**Fig. 2**). For example, growth hormone is known to access arcuate neurons that express growth hormone–releasing hormone (GHRH), acting as an autoinhibitory signal for GHRH release.

Two methods have been instrumental in characterizing the regulation of these neurons. The first is the analysis of POMC/CART and AgRP/NPY gene expression or c-Fos expression as a marker of neuronal activation. With the discovery of the adipostatic hormone leptin, attention focused on defining neural circuits responsible for mediating the leptin signal. POMC- and NPY neurons were identified as two neurochemically defined sites of expression of the leptin receptor in the CNS[24] and as sites where c-Fos is activated by both peripheral and central administration of leptin[25]. Even before studies on the effect of the leptin gene product on NPY, data suggested that aberrant regulation of orexigenic hypothalamic NPY was responsible for a component of the obesity syndrome in leptin-deficient mice[26]. This hypothesis was confirmed when it was demonstrated that crossing leptin-deficient ($Lep^{ob/ob}$) mice with NPY-null ($Npy^{-/-}$) mice reduced the obesity

of the $Lep^{ob/ob}$ mice by approximately 50% (ref. 27). Fasting potently upregulates levels of AgRP and NPY mRNA (5–10×) and modestly decreases POMC mRNA levels (20–50%), and leptin replacement to pre-fasting levels normalizes expression of these genes[28–30].

Before the discovery of leptin as the principal adipostatic hormone, extensive research on insulin demonstrated that this hormone has some adipostatic activity as well (for review, see ref. 31). Central administration of insulin reduces food intake and body weight, and notably, the arcuate nucleus is one of the prominent sites of insulin receptor expression. Like leptin, insulin upregulates arcuate NPY and AgRP expression and decreases arcuate POMC gene expression; it also activates ATP-sensitive K^+ channels in what are likely to be arcuate NPY neurons[32]. Although leptin and insulin activate different signal transduction pathways, both hormones seem to activate phosphatidylinositol 3′-kinase (PI3K) in the hypothalamus and seem to be dependent on PI3K for their anorexigenic activity. A mouse strain with a brain-specific insulin receptor knockout shows a mild obesity syndrome[33], arguing for a physiological role for central insulin action in energy homeostasis, and as described below, central insulin signaling also seems to be important for the regulation of glucose production by the liver[34].

Estrogen is another hormone that is likely to regulate POMC neurons in a manner relevant to energy homeostasis. The arcuate nucleus and VMH are major sites of estrogen receptor expression. POMC neurons are a presynaptic target of estrogens[35] and provide synaptic input to GnRH-expressing neurons. Estrogens can also modulate the activity of G protein–linked inwardly rectifying potassium channels (GIRKs) in POMC neurons[36]. Direct evidence has not yet been obtained, however, to determine whether the anorexigenic actions of estrogen depend on arcuate POMC neurons or on other elements of the central melanocortin system.

Electrophysiological recording using mouse hypothalamic slice preparations, in which the rare POMC/CART and NPY/AgRP cell types could be identified using either POMC-GFP or NPY-sapphire transgenes, has also provided a method for direct analyses of the regulation of these cell types. For example, one transgenic line was created using the hypothalamic POMC promoter driving expression of enhanced green fluorescent protein[37]. Colocalization studies using an antibody

against the POMC peptide β-endorphin showed that more than 99% of arcuate POMC neurons expressed detectable GFP. Whole-cell patch-clamp recordings and a loose cell-attached patch method using this system were then developed to characterize the responsiveness of these neurons to leptin and other agents[37–41]. All POMC neurons seem to show spontaneous action potentials, and leptin was found to inhibit the release of GABA from NPY terminals synapsing onto POMC neurons (**Fig. 2**); in addition, immunoelectron microscopy demonstrated that many POMC cell bodies are contacted by terminals containing both GABA and NPY[37]. In addition to characterization of leptin action, this preparation was also used to demonstrate that MC3R is an inhibitory autoreceptor on the POMC circuit[37]. Notably, levels of gene expression in the central melanocortin system reflect metabolic state, as does the electrical activity of the NPY/AgRP neurons, and this property is maintained in hypothalamic slices used for electrophysiological studies[42]. The spontaneous firing rate of the NPY/AgRP neuron is generally quite low (0.5 Hz) and is elevated threefold by fasting. The activation can be prevented by administration of leptin to the food-deprived animal.

The best evidence of a role for the hypothalamic melanocortin system in a direct response to acute signals of satiety and hunger comes from data on ghrelin. Identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR)[43,44], ghrelin is an acylated 28-amino-acid peptide predominantly secreted by the stomach, regulated by ingestion of nutrients[45–47] and having potent effects on appetite[47,48]. Ghrelin levels are markedly reduced with meal ingestion in both rodents and humans but rebound to baseline before the next meal and increase after an overnight fast[46–48]. GHSRs have been found on arcuate NPY neurons[49], and pharmacological doses of ghrelin injected peripherally or into the hypothalamus activate c-Fos solely in arcuate NPY neurons in rats[50]. Such doses also stimulate food intake and obesity, in part by stimulating NPY and AgRP expression[51–53], thus antagonizing leptin's anorexic effect[53]. On the cellular level, electrophysiological analyses suggest that ghrelin acts on the arcuate NPY/AgRP neurons to coordinately activate these orexigenic cells, while inhibiting the anorexigenic POMC cells by increasing GABA release onto them[38].

Ghrelin can also stimulate appetite and food intake in humans when given in a supraphysiological dose, but whether physiological changes in ghrelin levels or ghrelin signaling affect human energy homeostasis remains unknown. Ghrelin's characteristics make it unique among the gut-derived signals. Unlike other enteropancreatic signals involved with energy homeostasis, ghrelin secretion is inhibited in response to meals, and instead of acting as a satiety signal (like CCK or PYY_{3-36}), ghrelin stimulates appetite, potentially through arcuate signaling. These properties strongly suggest that ghrelin is a candidate 'meal-initiating' signal[47]. Much work needs to be done to establish the physiological role of ghrelin in meal initiation and body weight regulation and to establish its mechanism or mechanisms of action. Considerable evidence indicates that the melanocortin system is central to ghrelin's effects on food intake. Stimulation of food intake by ghrelin administration is blocked by administration of NPY receptor antagonists[53] and reduced in $Npy^{-/-}$ mice. Administration of the melanocortin agonist MTII blocks further stimulation of weight gain by growth hormone releasing peptide-2, a synthetic GHSR agonist, in $Npy^{-/-}$ mice[54]. Finally, peripheral administration of ghrelin activates c-Fos expression only in arcuate NPY/AgRP neurons, not in other hypothalamic or brainstem sites[55], and ablation of the arcuate nucleus blocks the actions of ghrelin administration on feeding but not elevation of growth hormone[56]. Despite activating c-Fos only in arcuate NPY neurons, peripheral ghrelin may access the arcuate nucleus through vagal afferents. GHSR is expressed on vagal afferents, and ghrelin suppresses firing of vagal nerves. Furthermore, surgical or chemical vagotomy blocks stimulation of feeding and
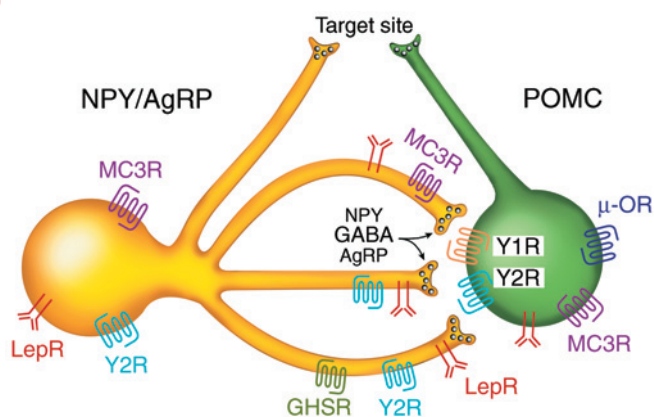
Figure 2 Schematic of the melanocortin system within the arcuate nucleus of the hypothalamus. NPY/AgRP and POMC neurons within the arcuate nucleus form a coordinately regulated network because of dense NPY/AgRP fibers projecting to POMC cell bodies. Some receptors for the large numbers of hormones and neuropeptides known to regulate the network are indicated. LepR, leptin receptor; μ-OR, μ opioid receptor; Y2R, type 2 NPY receptor. In most cases, whether the receptors are presynaptic or postsynaptic is not known. Figure modified from ref. 37.

c-Fos activation in the arcuate nucleus after peripheral but not central administration of ghrelin[57].

How can these findings be assembled into a single model? Peripheral ghrelin may largely suppress neurons in brainstem satiety centers, thus explaining the lack of c-Fos activation at these sites. The NTS sends dense catecholaminergic projections to the arcuate nucleus, so although a convergence of ascending vagal afferent information arrives at the arcuate nucleus from both the brainstem and other hypothalamic nuclei, it is possible that NTS neurons inhibited by ghrelin synapse directly with NPY arcuate neurons, thus explaining the absence of other hypothalamic neurons activated by peripheral ghrelin. Although the possibility is still controversial, there may be a novel set of ghrelin-positive neurons in the hypothalamus. These cells, identified by immunohistochemistry, make numerous connections with NPY fibers in the arcuate nucleus as well as with other neurons involved in energy homeostasis[38]. Little data exists about the regulation of hypothalamic ghrelin or about how information from these neurons is integrated with information derived from peripheral ghrelin, which is also known to act on the NPY/POMC arcuate network.

Peptide YY (PYY), a peptide related to NPY and pancreatic peptide, is released postprandially by endocrine cells in the ileum and colon[58]. PYY is found *in vivo* in both a full-length 36-amino-acid and a 34-amino-acid form (PYY_{3-36}), in an approximately 2:1 to 1:1 molar ratio[59]. PYY is a potent agonist of both Y1 and Y2 receptors, whereas PYY_{3-36} is a Y2-specific agonist, with an approximately 1,000-fold greater affinity for the Y2 than for the Y1 receptor. Recently, PYY_{3-36} was demonstrated to inhibit food intake acutely in rats in a Y2-dependent manner and to activate c-Fos in a small number (10–15%) of arcuate POMC neurons[60,61]. Initially, it was proposed that PYY_{3-36} might access arcuate Y2 receptor sites, altering food intake by activating arcuate POMC neurons. However, PYY_{3-36}-induced inhibition of food intake has been observed in both MC4R[60] and POMC[62] knockout mice, making this model less likely. Furthermore, peripheral administration of PYY_{3-36} also activates c-Fos in the brainstem and induces conditioned taste aversion, suggesting a mechanism of action independent of the central melanocortin system[63].

Nutrients and the hypothalamic melanocortin system

It has been proposed that the hypothalamus, in addition to responding to long-term adipostatic signals (leptin and insulin) and to acute satiety/hunger hormones (ghrelin) may also depend on a direct nutrient sensor to regulate food intake and energy balance. This concept is essentially an extension of the glucostatic hypothesis, which postulated that meal initiation may be regulated, in part, by central sensing of blood glucose levels[64]. For example, intracerebroventricular administration of oleic acid, a long-chain fatty acid, inhibits food intake and glucose production[65]. Furthermore, animals overfed a high-fat diet become resistant to the anorexigenic effects of oleic acid[66]. Additionally, central inhibition of the rate-limiting enzyme of fatty acid oxidation, carnitine palmitoyltransferase-1, also decreases food intake and glucose production[67]. These studies all involved intracerebroventricular administration, which allows access to both forebrain and hindbrain sites, so additional work will be needed to determine the sites where lipids or lipid oxidation might act as an energy sensor.

Another cellular energy sensor, the enzyme AMP kinase (AMPK), has also been recently been demonstrated to be involved in energy homeostasis[68,69]. Notably, the enzyme is not only a cellular energy sensor but is also inhibited in certain hypothalamic nuclei by leptin, insulin, melanocortin agonists, high glucose levels and refeeding. Furthermore, constitutively active AMPK blocks inhibition of feeding and weight loss induced by leptin. Although AMPK activity is inhibited in the arcuate nucleus and PVH by refeeding[69], exercise in rats seems not to alter the hypothalamic activity of the enzyme[70]. The expression and activity of AMPK in POMC, AgRP or MC4R neurons has not yet been reported, although the data above suggest that AMPK levels in the PVH may be mediated by MC4R agonists.

Thus far, one line of evidence shows directly that the melanocortin system might be a component of a hypothalamic nutrient sensor. POMC neurons express both Kir6.2 channel and sulfonylurea receptor-1 channel subunits and thus are likely to have a functional ATP-sensitive K^+ channel, a mechanism for linking neuronal activity to levels of cellular energy stores. Furthermore, the firing rate of the cells seems to be responsive to glucose concentrations in the bath[41]. Ultimately, formal proof of the physiological relevance of a nutrient sensor to hypothalamic control of energy homeostasis will require demonstrating several things: first, that hypothalamic systems such as the melanocortin system respond directly either to postprandial changes in blood nutrient levels or to chronic changes resulting from obesity or starvation; and second, that the degree of response is significant relative to responses to changes in leptin, ghrelin, insulin, glucocorticoids, CCK and other hormones that have evolved to communicate aspects of metabolic state to the CNS.

Neural inputs to the hypothalamic melanocortin system

It is also important to consider neural inputs to the arcuate and brainstem melanocortin-expressing neurons and inputs to MC3R and MC4R cells. It is likely that the melanocortin system interacts at one or more levels with a wide variety of other circuits involved in energy homeostasis, including those regulating motivated behaviors, circadian rhythm, reproductive function and olfaction. A fundamental aspect of these circuits is also the seemingly asymmetrical innervation of POMC neurons by a dense array of NPY/AgRP fibers derived from adjacent arcuate NPY/AgRP cells, as discussed above. As a consequence, the arcuate POMC and NPY/AgRP cell bodies constitute a functional unit in which neural inputs to NPY/AgRP cells may rapidly affect both NPY/AgRP and POMC neurons. Alternatively, it is possible to imagine inputs to arcuate POMC cells that do not alter the activity of the NPY/AgRP neurons.

Neural inputs to brainstem POMC neurons remain largely uncharacterized, although as described below, these cells do receive direct inputs from vagal afferent nerves. In the hypothalamus, α-adrenergic and serotonergic inputs to the melanocortin system have been characterized, although neither system has been well-characterized neuroanatomically. For example, POMC neurons express $5-HT_{2c}$ receptors and can be activated by dexfenfluramine, and inhibition of food intake by this compound in the mouse is blocked by the melanocortin antagonist SHU9119 (ref. 39). Serotonergic inputs to POMC neurons may also be important for the actions of cytokines on the melanocortin circuits during cachexia. Both arcuate NPY/AgRP and POMC neurons also receive innervation from orexin neurons originating in the LHA[71], and tachykinin-immunoreactive fibers synapsing onto arcuate NPY/AgRP cells have been characterized as well.

Anatomy and regulation of the brainstem melanocortin system

The brainstem is classically understood as the center for detection of and response to hunger and satiety signals. An alternate route for satiety factors and nutrients to activate the hypothalamic POMC neurons would involve the known action of these factors at the brainstem, as sites of vagal afferent action and gut peptide action involve brainstem cell groups such as the NTS that send dense projections to mediobasal hypothalamic cell groups like the arcuate nucleus.

The NTS is the primary site for innervation by vagal afferents from the gut (for review, see ref. 72). The afferent branches deriving from

different aspects of the gastrointestinal tract map viscerotopically along the NTS, from its rostral to its caudal aspect[73]. Rostrally, the NTS is a bilaterally symmetrical nucleus that merges into a single medial body at its caudal extent, called the commissural NTS[74]. Vagal afferents deriving input from the upper gastrointestinal tract respond to three basic stimuli: gastric and duodenal distension or contraction, chemical contents of the lumen and gut peptides and neurotransmitters released from the stomach and duodenum in response to nutrients[72]. In the rat, vagal afferents responding to gastric and duodenal distension tend to map to the medial and commissural divisions of the NTS[75,76]. CCK vagal afferents also map to the caudal NTS[77]. The dorsal motor nucleus of the vagus, located just ventral to the NTS, is the primary site of motor efferents to the gut and is densely innervated by NTS fibers. Together, these cell groups form the dorsal vagal complex (DVC) and serve as the neuroanatomical substrate for the vago-vagal reflex.

Several lines of evidence suggest an important role for melanocortin signaling within the brainstem in the satiety and vago-vagal reflexes. First, a discrete site of POMC expression in the brainstem (**Fig. 3**) has been identified in the commissural NTS[78,79]. ACTH, α-MSH and β-endorphin immunoreactivity have been identified in these cells[79], and deafferentation of the hypothalamic POMC-expressing fibers using knife cuts[19] or treatment with monosodium glutamate[20] indicate that the arcuate POMC fibers exclusively innervate forebrain, midbrain and periaquaductal gray matter. POMC NTS fibers are found in many sites in the caudal mesencephalon and spinal cord, with sites of dual innervation by hypothalamic and brainstem POMC fibers seen in the locus coeruleus, parabrachial nucleus, rostral NTS, dorsal motor nucleus of the vagus and lateral reticular nucleus. MC4R mRNA is also expressed in multiple sites within the brainstem, with quite high levels within the DMV, NTS and parabrachial nucleus[80,81]. Fourth-ventricular[82] and parenchymal DVC injections[83] of melanocortin agonists and antagonists produce effects on both food intake and weight gain comparable to those seen with lateral ventricular injections. These data imply a marked coordination between the brainstem and hypothalamic melanocortin systems.

In addition to signals from gut distension, gut peptides stimulated by meal intake mediate satiety through centers in the brainstem. These signals then are thought to interact primarily with centers of long-term weight regulation through neural connections to the hypothalamus to regulate total daily intake by adjusting meal size, number of meals or both.

Cholecystokinin is a good example of such a gut peptide. Produced by the gastrointestinal tract in response to meal ingestion, CCK's diverse actions include stimulation of pancreatic enzyme secretion and intestinal motility, inhibition of gastric motility and acute inhibition of feeding. Early experiments involving peripheral administration of CCK supported a role for increased CCK levels in the early termination of a meal[84]. The finding that repeated injections of CCK lead to reduced meal size without a change in body weight, because of a compensatory increase in meal frequency, argued against CCK acting as a signal regulating long-term body weight[85]. Studies using CCK receptor–specific antagonists as well as surgical or chemical vagotomy have shown that the effects of CCK on satiety are specifically mediated by one type of CCK receptor, CCKAR, on afferent vagal nerves[86,87].

This interaction between acute vagal input from CCKA receptors and body weight set point is primarily mediated by neural connections to the hypothalamus; this interaction is affected by insulin and leptin signals and inputs from the hindbrain, which receives input from vagal afferents. Indeed, central administration of insulin and leptin potentiates the satiety-inducing effects of peripherally administered CCK[88,89]
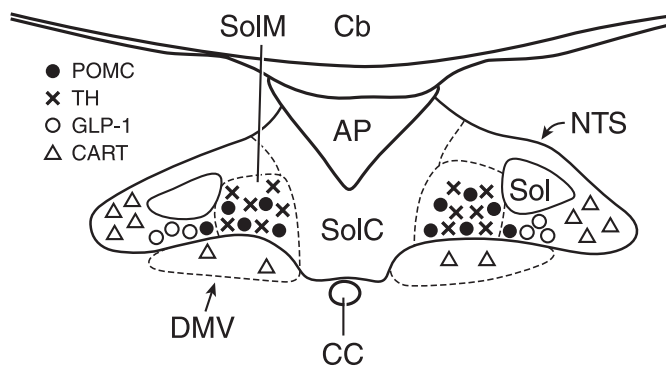
Figure 3 Distribution of POMC neurons within the nucleus tractus solitarius of the brainstem. POMC expression defines a unique population of TH-negative, GLP-1–negative cells within the medial nucleus of the NTS. AP, area postrema; Cb, cerebellum; CC, central canal; DMV, dorsal motor nucleus of the vagus; Sol, solitary tract; SolC, commissural nucleus of the solitary tract.

and, with repeated injections, leads to a sustained weight loss greater than that resulting from injection of the agents separately[90].

Although basomedial hypothalamic cell groups involved in leptin signaling are densely innervated by catecholaminergic neurons from the brainstem, a recent report demonstrates that norepinephrine is not required for CCK-induced reduction of feeding, as the dopamine β-hydroxylase knockout mouse is still responsive to CCK-induced satiety[91]. However, the brainstem POMC neurons may be one of the non-catecholaminergic cell groups involved in transmitting CCK's satiety signal. Approximately 30% of the POMC NTS neurons are activated, as determined by induction of c-Fos immunoreactivity, after intraperitoneal administration of a dose of CCK that initiates satiety[92]. Furthermore, the central melanocortin system as a whole is clearly important for the satiety effect mediated by CCK, as $Mc4r^{-/-}$ mice are largely resistant to CCK-induced satiety, and fourth-ventricular administration of the melanocortin antagonist SHU9119 seems more potent than third-ventricular administration in blocking the actions of CCK[92]. Only a very small fraction of cells activated in the brainstem by CCK-induced satiety are POMC NTS cells. Thus, whereas CCK-mediated satiety seems dependent on MC4R signaling, it is unlikely to be dependent on the POMC NTS cells. The regulation of POMC NTS cells by CCK may be the first report of regulation of these cells: feeding-induced satiety also produces upregulation of c-Fos in 10–15% of these cells[92]. Finally, the POMC NTS cells are also activated by leptin (K.L.J. Ellacott and R.D.C., personal communication). Approximately 50% of the cells showed induction of c-Fos immunoreactivity after peripheral leptin treatment, and approximately 30% of all c-Fos–immunoreactive cells in the NTS after leptin treatment were POMC-GFP positive. Although α-MSH and β-endorphin immunoreactivity were observed in the commissural NTS in early reports characterizing these cells in the rat, these peptides seem to be expressed at low levels in the brainstem, and achieving these results is technically challenging. The availability of POMC-GFP mouse strains has facilitated all the recent studies of the regulation and neurochemical identity of these cells, but a more detailed analysis of their POMC peptide content and regulation remains an important goal. Using POMC-GFP immunoreactivity as a marker, these cells have been demonstrated to be CART-negative, tyrosine hydroxylase (TH)-negative and glucagon-like peptide (GLP)-1–negative NTS neurons[92]. Thus, they represent a previously unknown class of NTS neurons regulated by both leptin and acute satiety signals. They have also been shown to be innervated by vagal afferents[93].

Additional roles for the central melanocortin system

The melanocortin system is probably the best-characterized CNS system involved in the regulation of energy homeostasis, and a number of animal experiments suggest potential therapeutic applications of melanocortin agonists and antagonists in the treatment of obesity and diabetes, as well as cachexia, respectively. With this in mind, it is important to remember that the POMC and NPY/AgRP neurons project to a wide array of brain nuclei, with MC4R alone being identified in over 100 sites[80]. Not unexpectedly, administration of melanocortins has been shown to have physiological effects beyond the regulation of food intake and energy expenditure, including effects on blood pressure, heart rate, inflammation, natriuresis and erectile function.

The actions of melanocortins on the cardiovascular system are complex and involve multiple receptors. Central or intracarotid administration of the MC3R-specific agonist γ-MSH has pressor and cardioacceleratory effects, although the pressor activity of the peptide does not seem to require MC3R, implicating an uncharacterized receptor. Hypotensive and bradycardic responses to electrical stimulation of the arcuate nucleus can be blocked by administration of the melanocortin antagonist SHU9119 (ref. 94) within the DVC, suggesting a role for brainstem MC4R in cardiovascular control. The central melanocortin system also influences inflammation: administration of α-MSH has potent anti-inflammatory effects that are dependent on intact autonomic outflow, perhaps related to the control of vascular permeability (for review, see ref. 95). The physiological relevance of these findings is not yet clear, although ACTH and α-MSH have resuscitative effects in models of hemorrhagic shock.

The melanocortin system also is involved in natriuresis[96]. γ-MSH stimulates natriuresis through kidney MC3R (ref. 97), and both γ-MSH–deficient and MC3R knockout mice develop salt-sensitive hypertension[98]. In γ-MSH–deficient prohormone convertase-2 knockout mice, central infusion of γ-MSH corrects the salt-sensitive hypertension, implicating central MC3R sites in this pathway as well.

Central melanocortin receptors are also implicated in the control of sexual function, stimulating lordosis in female rats and erectile activity in male rodents as well as in humans[99]. Both central and peripheral MC4R are implicated in erectile function, and MC3R is implicated in lordosis. Melanocortins have been tested in clinical trials for the treatment of erectile dysfunction[100].

Conclusions

The central melanocortin system is a fascinating collection of neural circuits that provides an ideal neuroanatomical substrate for the integration of long-term adipostatic signals from leptin with acute hunger and satiety signals from vagal afferent activity, gut satiety and hunger peptides, and nutrients. The positioning of POMC and NPY/AgRP cell bodies adjacent to the median eminence, and of brainstem POMC cell bodies adjacent to the area postrema, imply that the system may be sampling blood-borne hormone and nutrient concentrations. There are reciprocal projections from brainstem and hypothalamic sites of melanocortin cell bodies to integrative and motor centers with high densities of MC4R expression, such as the PVH and DMV. This argues that the system is ultimately important in integrating many afferent inputs with behavioral and autonomic responses that adjust energy intake and expenditure, thus maintaining energy homeostasis. Although the regulation of POMC and NPY/AgRP neurons has received considerable attention, understanding of the nature and regulation of MC3R and MC4R neurons remains in its infancy. For example, efferent pathways downstream of the melanocortin system have been only partially characterized. Likewise, few details exist concerning the mechanisms by which the melanocortin system stores information regarding levels of energy stores and integrates this information with output to affect levels of intake and expenditure. The central melanocortin system has potential as a site for therapeutic intervention for disorders such as obesity, diabetes and cachexia. However, to avoid potential side effects, it will also be important to learn more about the possible roles for these circuits in cardiovascular function, natriuresis, inflammation and sexual function.

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