The Central Melanocortin System and the Integration of Short- and Long-term Regulators of Energy Homeostasis

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ABSTRACT

The importance of the central melanocortin system in the regulation of energy balance is highlighted by studies in transgenic animals and humans with defects in this system. Mice that are engineered to be deficient for the melanocortin-4 receptor (MC4R) or pro-opiomelanocortin (POMC) and those that overexpress agouti or agouti-related protein (AgRP) all have a characteristic obese phenotype typified by hyperphagia, increased linear growth, and metabolic defects. Similar attributes are seen in humans with haploinsufficiency of the MC4R. The central melanocortin system modulates energy homeostasis through the actions of the agonist, α-melanocyte-stimulating hormone (α-MSH), a POMC cleavage product, and the endogenous antagonist AgRP on the MC3R and MC4R. POMC is expressed at only two locations in the brain: the arcuate nucleus of the hypothalamus (ARC) and the nucleus of the tractus solitarius (NTS) of the brainstem. This chapter will discuss these two populations of POMC neurons and their contribution to energy homeostasis. We will examine the involvement of the central melanocortin system in the incorporation of information from the adipostatic hormone leptin and acute hunger and satiety factors such as peptide YY ($PYY_{3–36}$) and ghrelin via a neuronal network involving POMC/cocaine and amphetamine-related transcript (CART) and neuropeptide Y (NPY)/AgRP neurons. We will discuss evidence for the existence of a similar network of neurons in the NTS and propose a model by which this information from the ARC and NTS centers may be integrated directly or via adipostatic centers such as the paraventricular nucleus of the hypothalamus (PVH).

I. Introduction

Pro-opiomelanocortin (POMC) modulates energy homeostasis principally through one of its cleavage products, α-melanocyte-stimulating hormone (α-MSH), which exerts a tonic inhibitory control on food intake and energy storage though its actions in the central nervous system (CNS) at two of the five known melanocortin receptors, melanocortin-3 receptor (MC3R) and melanocortin-4 receptor (MC4R) (for a review, see Cone, 1999). While the contribution of the agonist α-MSH is important, it is the endogenous antagonist at these receptors, agouti-related protein (AgRP) (Ollmann *et al.*, 1997), whose mRNA shows a greater degree of regulation by extremes of negative or positive energy balance such as fasting and diet-induced obesity in rodents (Mizuno and Mobbs, 1999;

Ziotopoulou *et al.*, 2000). The most-compelling evidence, however, for a pivotal role for the central melanocortin system in the regulation of energy homeostasis comes from studies in transgenic mice (for a review, see Butler and Cone, 2001). POMC and MC4R knockout mice and mice that overexpress the agouti gene (A^y/a) or AgRP all have a characteristic obese phenotype typified by hyperphagia, increased linear growth, and metabolic defects (Yen *et al.*, 1994; Huszar *et al.*, 1997; Ollmann *et al.*, 1997; Yaswen *et al.*, 1999). Similar attributes are seen in humans with mutations in genes of the central melanocortin system. Defects in the MC4R gene have been linked to obesity, particularly severe early-onset obesity in children (Farooqi *et al.*, 2000). Significantly, alterations in this gene have been linked to up to 5% of cases in children and adults (Farooqi *et al.*, 2003).

Although there are five melanocortin receptors, it is the MC3R and MC4R subtypes that have been implicated in the regulation of energy balance (for a review, see Adan *et al.*, 1997). While these receptors both have a fairly widespread distribution in the rodent brain (Roselli-Rehfuss *et al.*, 1993; Mountjoy *et al.*, 1994; Kishi *et al.*, 2003), POMC has a limited distribution, with only two neuronal populations described: one in the arcuate nucleus of the hypothalamus (ARC) and the other in the nucleus of the tractus solitarius (NTS) of the brainstem (Joseph *et al.*, 1983; Palkovits *et al.*, 1987; Bronstein *et al.*, 1992). Of these two populations, the ARC neurons have drawn the most attention from researchers. The ARC and other hypothalamic nuclei have classically been associated with the actions of leptin and the regulation of body weight in the long term, while the NTS and other brainstem nuclei predominantly are linked to the regulation of meal initiation and termination (Grill and Kaplan, 2002). We will review evidence for the involvement of both populations of POMC neurons in the regulation of energy homeostasis, both in the long term and short term, and discuss the potential for the integration of information from these two sites by adipostatic centers.

II. POMC Neurons and the ARC Neuronal Network

A. THE ARC NEURONAL NETWORK AND LONG-TERM REGULATORS OF ENERGY HOMEOSTASIS

The POMC neurons of the ARC are known to be responsive to leptin via leptin receptors (Ob-R) expressed on their surface (Cheung *et al.*, 1997). In addition to the POMC neurons, another important element of the melanocortin system in the hypothalamus is the neurons that express the melanocortin receptor antagonist AgRP, which also express the orexigenic peptide, neuropeptide Y (NPY) (Hahn *et al.*, 1998) and are leptin sensitive (Wilson *et al.*, 1999). These NPY/AgRP-containing neurons are able to form synapses with POMC neurons

of the ARC and exert regulatory effects, producing a neuronal network that is responsive to the modulatory actions of leptin (Figure 1) (Cowley *et al.*, 2001). In this model, leptin causes hyperpolarization of NPY/AgRP neurons, leading to a reduction in the release of gamma aminobutyric acid (GABA) that, in turn, causes disinhibition of the POMC neurons with which they synapse. In addition to its indirect actions on the POMC neurons via NPY/AgRP cells, leptin appears to act on the POMC system directly by causing a depolarization of the ARC neurons, increasing their firing rate. This model demonstrates how leptin may serve as an overall modulator of energy homeostasis by altering the firing rate of orexigenic and anorexigenic neurons. The fact that serum leptin levels do not vary after meals (Korbonits *et al.*, 1997) but generally are proportional to adipose mass (Maffei *et al.*, 1995) suggests that leptin is not acting as an anorectic factor

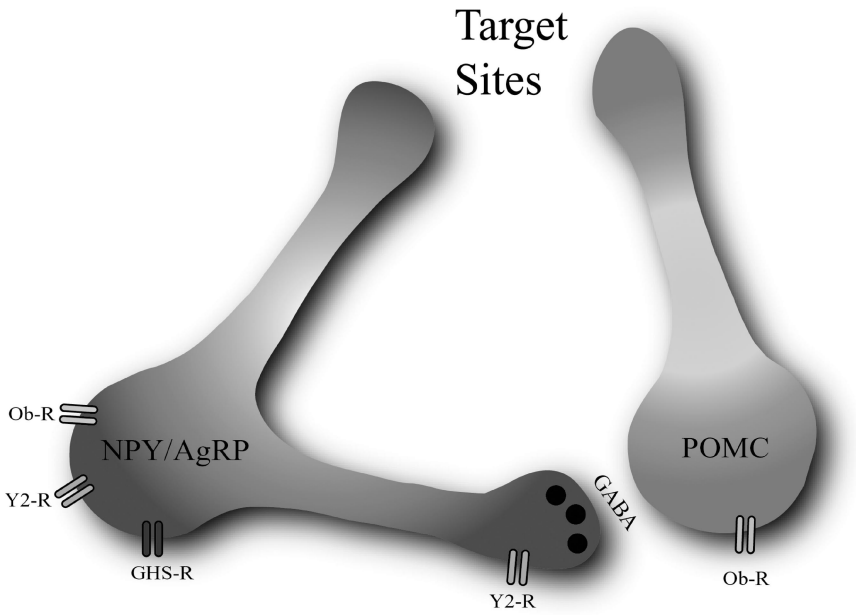

FIG. 1. The arcuate nucleus (ARC) neuronal network. Neuropeptide Y/agouti-related protein (NPY/AgRP) neurons form synapses with the pro-opiomelanocortin/cocaine and amphetamine-regulated transcript (POMC/CART) neurons in the ARC, forming a regulatory network that is responsive to leptin via leptin receptors (Ob-R) present on their surface. Leptin acts on POMC neurons directly and indirectly via a reduction in the release of gamma aminobutyric acid (GABA) from NPY/AgRP neurons. The circuit is able to respond, via growth hormone secretagogue receptor (GHS-R) and Y2-R, to signals from ghrelin and $PYY_{3–36}$.

but rather as an indicator of the long-term energy status of the animal. Thus, modulation of the firing rate of neurons of the ARC and other hypothalamic sites may be a means by which the body weight of an animal is maintained and adjusted over extended periods of time in response to variations in leptin levels.

B. THE MELANOCORTIN SYSTEM AND SHORT-TERM REGULATORS OF ENERGY HOMEOSTASIS

In addition to the mediation of long-term changes in energy balance via signals from leptin, we have shown that POMC neurons of the ARC may be able to respond to signals from the gut hormone peptide $YY_{3–36}$ ($PYY_{3–36}$) (Batterham *et al.*, 2002). $PYY_{3–36}$, an N-terminal truncated form of PYY, is released from the lower intestine following a meal in proportion to the number of calories ingested (Pedersen-Bjergaard *et al.*, 1996). ARC POMC neurons are activated by administration of $PYY_{3–36}$ via Y2 receptors (Y2-R) on NPY/AgRP neurons that, in turn, causes modulation of the hypothalamic ARC network previously described. This evidence suggests that the ARC network actually may integrate information from both long-term signals of nutritional status and satiety signals that are released postprandially from the gut. While the finding that direct injection of $PYY_{3–36}$ into the ARC causes a reduction in food intake, it remains to be established whether the ARC is the functional site of action of $PYY_{3–36}$ *in vivo* or whether, in common with other postprandially released gastric peptides, it acts via the brainstem and sites in the gut itself. In addition to $PYY_{3–36}$, there is evidence to suggest that the network may be activated by other gut hormones. The satiety signal cholecystokinin (CCK) has been shown to electrically (Burdakov and Ashcroft, 2002) modulate the activity of ARC neurons, although these have not been identified as containing POMC or NPY/AgRP. This indicates that there is potential for an interaction between the central melanocortin system and other postprandially released gut peptides.

Another more-recently identified gut-derived peptide is ghrelin. Ghrelin is the endogenous peptide for the growth hormone secretagogue receptor (GHS-R), the mRNA for which is expressed in a number of sites in the hypothalamus (Guan *et al.*, 1997). Ghrelin originally was described as being produced by the oxyntic cells of the stomach (Kojima *et al.*, 1999) but since has been shown to be expressed at low levels in the small intestine (Date *et al.*, 2000), kidney (Mori *et al.*, 2000), testis (Tanaka *et al.*, 2001), placenta (Gualillo *et al.*, 2001), brain (Lu *et al.*, 2002; Cowley *et al.*, 2003), lymphocytes (Hattori *et al.*, 2001), pituitary (Korbonits *et al.*, 2001), and pancreas (Volante *et al.*, 2002). Perhaps unsurprisingly, due to the close association between growth and energy homeostasis and what was already known about the effects of synthetic growth hormone secretagogue (Bercu *et al.*, 1992), ghrelin peptide and mRNA levels were shown to be

regulated by changes in energy balance such as fasting (Tschop *et al.*, 2000; Cummings *et al.*, 2001), hypoglycemia (Toshinai *et al.*, 2001), and diet-induced obesity (Tschop *et al.*, 2000) in rodents. However, the effects of ghrelin are independent of GH secretion (Tschop *et al.*, 2000; Wren *et al.*, 2000; Nakazato *et al.*, 2001).

NPY/AgRP neurons of the ARC have been implicated in mediating ghrelin's effects on energy homeostasis (Kamegai *et al.*, 2001; Nakazato *et al.*, 2001; Shintani *et al.*, 2001; Lawrence *et al.*, 2002b; Wang *et al.*, 2002). Ghrelin has a unique distribution in the brain, encompassing the internuclear space between the ARC, ventromedial (VMH), dorsomedial (DMH), and paraventricular hypothalamic nuclei (PVH) (Cowley *et al.*, 2003). The discovery of this network led to questions about whether the effects of ghrelin on the ARC NPY/AgRP neurons were due to centrally or peripherally derived ghrelin, or both. Indeed, axons from ghrelin-containing neurons form synaptic contact with NPY/AgRP and POMC neurons of the ARC (Cowley *et al.*, 2003). Electrical recording from these ARC neurons indicates that ghrelin is able to cause depolarization of ARC NPY/AgRP neurons and hyperpolarization of POMC neurons. When considered in conjunction with studies showing that c-fos is activated in NPY/AgRP but not in POMC neurons following peripheral ghrelin administration (Wang *et al.*, 2002), the data would suggest that the effect of ghrelin on POMC neurons is probably inhibitory, mediated by the action of GABA released by NPY/AgRP neurons (Cowley *et al.*, 2003).

In the same study, it was demonstrated that in addition to its effects in the ARC, ghrelin is able to influence the activity of PVH corticotropin-releasing hormone (CRH) neurons, possibly via an increase in release of GABA from NPY/AgRP neurons, in a similar manner to its effects on POMC neurons. The interaction between the ARC neuronal network and the neurons of the PVH will be discussed further in this review. In addition to interacting with NPY and the central melanocortin system, evidence suggests that ghrelin interacts with the orexigenic peptide orexin/hypocretin in the brain. Central administration of ghrelin in rats causes activation of orexin-containing neurons of the lateral hypothalamic area (LHA) (Lawrence *et al.*, 2002b). Ghrelin-immunoreactive terminals make contact with orexin neurons in the LHA (Toshinai *et al.*, 2003). The blockade of orexin-A and -B receptors by injection of antisera attenuates the effects of centrally administered ghrelin on food intake, providing *in vivo* evidence for an interaction between the two peptides. The wide distribution of ghrelin-immunoreactive neurons (Cowley *et al.*, 2003), GHS-R mRNA (Guan *et al.*, 1997), and the data outlined earlier suggest that, in common with leptin, ghrelin may serve as an overall modulator of a number of anorexigenic and orexigenic pathways directly and via the ARC neuronal network.

III. POMC Neurons of the NTS

A. THE INVOLVEMENT OF NTS POMC NEURONS IN THE REGULATION OF FOOD INTAKE

In recent years, while most of the attention in the field of energy homeostasis has been concentrated on the hypothalamus, the importance of the brainstem largely has been neglected. As such, comparatively little is known about the POMC neurons of the brainstem. An extensive network of fibers immunoreactive for POMC-derived peptides exists in the brainstem. This network includes immunoreactivity in the NTS, lateral reticular nucleus (A5-C1 groups), ventrolateral medulla (A1 cell group), and nucleus ambiguus. The only POMC cell bodies present in the brainstem are found in the commissural region of the NTS. Interestingly, POMC neurons have been shown to send a number of projections within the brainstem, particularly to the ventral lateral medulla and onto the spinal cord. However, studies involving lesioning of hypothalamic connections indicate that only 30–50% of the POMC-derived immunoreactivity in the brainstem originates from cell bodies in the commissural NTS (Palkovits *et al.*, 1987; Joseph and Michael, 1988). The remainder of the immunoreactivity seen is derived from projections from the hypothalamic POMC neurons. Hypothalamic POMC fibers innervate the brainstem via two distinct pathways: one that travels via the periaqueductal gray and the dorsomedial tegmentum to innervate the rostral NTS and lateral reticular nucleus (A5-C1 groups) and a second, more-dominant pathway through the ventrolateral tegmentum, believed to be the route of the majority of descending pathways, that innervates the rostral NTS, ventrolateral medulla (A1 cell group), nucleus ambiguus, and the descending spinal bundle. The MC4R is expressed at a number of these sites, indicating that hypothalamic POMC may exert some of its effects on energy homeostasis via receptors in the brainstem (Kishi *et al.*, 2003).

The work of Grill and colleagues has demonstrated that the melanocortin system of the brainstem plays a role in regulation of energy homeostasis. Administration of MTII, a synthetic melanocortin receptor agonist, or SHU9119, an MC3R and MC4R antagonist, into the fourth ventricle or directly into the dorsal vagal complex (DMX) causes a reduction in food intake in the case of MTII and an increase in food intake in the case of SHU9119 (Williams *et al.*, 2000). Following fourth ventricular administration, changes seen are comparable with those following administration of MTII or SHU9119 into the lateral ventricle (Grill *et al.*, 1998).

A potentially important consideration when studying the melanocortin system of the brainstem is the low level of expression of the endogenous antagonist AgRP. In contrast to the hypothalamus, where there is a relatively high level of expression of both the AgRP- and POMC-immunoreactive fibers and terminals

that project to identical areas of the brain (Bagnol *et al.*, 1999), the brainstem has few, if any, AgRP-immunoreactive cell bodies and receives limited terminals from the ARC. Given the lack of AgRP in the brainstem, it is unknown what regulates melanocortinergic tone in this area. It is unlikely that much regulation comes from AgRP projections from the hypothalamus but it is feasible that the system in this area is regulated by other mechanisms such as differences in POMC processing or post-translational modification (for a review of POMC processing, see Pritchard *et al.*, 2002).

B. EVIDENCE FOR THE EXISTENCE OF A REGULATORY NEURONAL NETWORK IN THE BRAINSTEM: COMPARISON WITH THE ARC NEURONAL NETWORK

Taking into account all the evidence to suggest the existence of a POMC-NPY/AgRP neuronal network in the ARC, it is interesting to speculate whether a similar network may be involved in modulating energy homeostasis via the brainstem. While the ARC network is able to respond to what are considered long-term as well as short-term modulators of energy homeostasis, is there any reason that a similar network should not exist in the NTS?

A number of similarities between the ARC and the NTS make the existence of such a network possible. First, they both lie in close anatomical proximity to a circumventricular organ, the median eminence in the case of the ARC and the area postrema in the case of the NTS. Although AgRP cell bodies are absent, the NTS contains cell bodies that show immunoreactivity for NPY and POMC-derived peptides. In common with the ARC, the NTS contains leptin receptors (Hakansson *et al.*, 1998; Mercer *et al.*, 1998). These neurons have been shown to be able to mediate the inhibitory effects of leptin on food intake and body weight gain following fourth ventricle administration (Grill *et al.*, 2002). In addition to causing activation of hypothalamic sites, peripheral administration of leptin activates neurons of the NTS, as measured by the expression of signal transducer and activator of transcription (STAT)-3 (Hosoi *et al.*, 2002; Munzberg *et al.*, 2003) or c-fos (Elmquist *et al.*, 1997). The NTS receives projections from numerous centers in the brain but is also the site at which vagal afferents terminate, making it an important site in mediating the vago-vagal reflex.

IV. The PVH as a Site of Integration of Hypothalamic and Brainstem Signals

The PVH is an important hypothalamic nucleus in the integration of autonomic and neuroendocrine information (for a review, see Palkovits, 1999). The PVH receives projections from a number of sites in the brain, including the ARC and NTS (Sawchenko and Swanson, 1983). Both melanocortin and NPY/

AgRP terminals are present in this area (Bagnol *et al.*, 1999). Indeed, NTS NPY neurons have been shown to project to the PVH (Sawchenko *et al.*, 1985). The PVH may serve as a site of integration of information from melanocortin, NPY/AgRP, and possibly other orexigenic and anorexigenic neurons via GABAergic interneurons. Evidence for this model comes from *in vivo* and electrophysiological studies. Direct injection of the melanocortin agonist MTII into the PVH results in a reduction in food intake. MTII at this site is able to functionally antagonize the orexigenic effects of NPY, indicating the potential for interactions between the two systems in the PVH *in vivo*. Electrophysiological studies have shown that neurons expressing NPY/AgRP and POMC have opposing actions on neurons of the medial PVH, potentiating and inhibiting GABAergic currents, respectively (Cowley *et al.*, 1999). Modulation of the central melanocortin system following intracerebroventricular administration of MTII, α-MSH, or AgRP activates a number of hypothalamic and extrahypothalamic sites in rats, including in the PVH (Thiele *et al.*, 1998; McMinn *et al.*, 2000; Hagan *et al.*, 2001). Indeed, the PVH seems to be a site that is activated following administration of a number of orexigenic and anorexigenic peptides, reinforcing the hypothesis that it is a key site for the integration of information regarding energy homeostasis (Hamamura *et al.*, 1991; Lambert *et al.*, 1995; Van Dijk *et al.*, 1996; Elmquist *et al.*, 1997; Edwards *et al.*, 1999; Lawrence *et al.*, 2002a).

The ARC melanocortin and NPY neurons innervate neurosecretory neurons of both the parvocellular and magnocellular subdivisions of the PVH (Piekut, 1985,1987; Liposits *et al.*, 1988; Sawchenko and Pfeiffer, 1988; Li *et al.*, 2000). The innervation of the thyrotrophin-releasing hormone (TRH) neurons has been particularly well characterized. Neurons containing immunoreactivity for both AgRP and NPY or α-MSH innervate TRH neurons in the PVH directly through projections from the ARC and indirectly via projections from the medial preoptic nucleus (Legradi and Lechen, 1999; Fekete *et al.*, 2000; Kawano and Masuko, 2000). These and numerous other anatomical studies highlight the importance of the PVH as a site for the integration of information from a number of systems and demonstrate how the regulation of energy balance may modulate other neuroendocrine processes such as the growth, reproductive, and stress axes (Schioth and Watanobe, 2002; Smith and Grove, 2002).

As discussed earlier, in addition to the PVH acting as a site of integration, the neurons of the ARC and NTS may communicate via direct projections between the two sites. ARC POMC neurons have been shown to project to a number of sites in the brainstem, including the NTS, periaqueductal gray, dorsal raphe nucleus, nucleus raphe magnus, nucleus raphe pallidus, locus coeruleus, parabrachial nucleus, nucleus reticularis gigantocellularis, and DMX (Chronwall, 1985; Sim and Joseph, 1991). Many of these regions contain MC4R mRNA (Mountjoy *et al.*, 1994; Kishi *et al.*, 2003), raising the possibility that these

receptors in the brainstem may be served by projections from the ARC neurons in addition to or in place of projections from the POMC neurons of NTS.

V. Summary

The evidence presented herein reinforces the importance of the POMC-NPY/AgRP system in the regulation of energy homeostasis and a number of other neuroendocrine processes. Localization of the POMC-NPY/AgRP neuronal networks in the ARC and possibly the NTS and the diversity of their neuronal projections from these sites make them well placed to respond to and coordinate both long-term adipostatic and short-term hunger/satiety signals between the periphery and the brain.

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